The Multinational Coordinated *Arabidopsis thaliana* Genome Research Project

Progress Report: Year Five

December, 1995

PREFACE

The "Multinational Coordinated *Arabidopsis thaliana* Genome Research Project" was established in 1990 to promote international cooperation in basic and applied research with *Arabidopsis*, a model plant species amenable to experimental manipulation in the laboratory. The primary objective of this project has been to understand the molecular basis of plant growth and development and to address fundamental questions in plant physiology, biochemistry, cell biology, and pathology. Initial plans were outlined in a publication (NSF #90-80) drafted five years ago by an ad hoc committee of nine scientists from the United States, Europe, Japan, and Australia. In recent years, this project has become a model for widespread participation and effective coordination of multinational research efforts in modern biology.

Arabidopsis thaliana, a small plant in the mustard family, was chosen for this large-scale research effort because it offers many advantages for detailed genetic and molecular studies. Among these features are its small size, short life cycle, small genome, ability to be transformed, availability of numerous mutations, and prolific seed production. By concentrating research efforts on a single model organism, detailed information on specific genes and cellular processes can be readily obtained and rapidly applied to a wide range of plants relevant to agriculture, health, energy, manufacturing, and the environment.

Each year since 1990, the scientific steering committee for the *Arabidopsis* Genome Project has prepared a progress report summarizing recent advances in *Arabidopsis* research. This is the fifth annual progress report published by the multinational steering committee in conjunction with the US National Science Foundation. Last year's report was an in-depth color brochure designed to explain the value and significance of *Arabidopsis* research to a wide audience. Anyone interested in learning more about *Arabidopsis* research should request a copy of that report from NSF (publication 95-43) or view an electronic version available on the Internet at http://www.nsf.gov/bio/pubs/arabid/start.htm.

In order to avoid duplication of effort, the purpose of this year's report is to present a concise overview of the current status of *Arabidopsis* research worldwide and to provide updated technical information relevant to the *Arabidopsis* research community. Additional information can be obtained through recent publications, electronic news groups and databases, and biological resource centers as noted in the text of this report. Multinational cooperation and communication continue to be an important feature of the *Arabidopsis* genome project. A brief overview of *Arabidopsis* research efforts in a number of participating countries is therefore included in this report. As with any document that attempts to summarize the contributions of many individuals, this report may fail to include or misrepresent some significant achievements. The steering committee hopes that members of the *Arabidopsis* community will overlook such shortcomings and will communicate any concerns to committee members so that future reports will be as accurate as possible.

We thank members of the *Arabidopsis* community, especially those listed here, for their contributions to this report: R. Amasino, M. Anderson, F. Ausubel, P. Benfey, M. Bevan, B. Buchanan, M. Cherry, J. Chory, J. Dangl, K. Davis, C. Dean, X.-W. Deng, J. Ecker, M. Estelle, D. Flanders, S. Kay, M. Koornneef, D. Kristofferson, B. Lemieux, E. Meyerowitz, F. Migliaccio, H.G. Nam, T. Newman, D. Preuss, J. Putterill, N. Raikhel, R. Schmidt, R. Scholl, D. Smyth, C. Somerville, N. Terryn, and D. Weigel.The Multinational Science Steering Committee

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EXECUTIVE SUMMARY

Remarkable progress has been made over the past five years towards meeting the objectives initially outlined in "A Long-Range Plan for the Multinational Coordinated *Arabidopsis thaliana* Genome Research Project". In just the past year, significant advances continued to be made in understanding the structure and function of the *Arabidopsis* genome, identifying and characterizing informative mutations, addressing a wide range of fundamental questions in plant biology, expanding biological resource materials, enhancing existing databases and communications networks, and applying knowledge gained through research with *Arabidopsis* to other organisms. Examples of these advances are highlighted in this progress report. As evidenced in the summary table below, *Arabidopsis* research continues to expand at a rate considered improbable just 10 years ago. This effort has truly been international in nature. Over 35 countries are presently involved in studies with *Arabidopsis*, and a large number of collaborative research networks have been established worldwide.

Current Status of *Arabidopsis* Research

Estimated genome size	100Mb
Estimated maximal number of genes	20,000
Approximate length of average gene	3 Kb
Contiguous genomic sequence determined to date	~ 1 Mb
Target for genomic sequence completed by 1998	20 Mb
Target date for completion of genomic sequence	2004
Entries for nuclear genes in GenBank(10/15/95)	>22,000
Partial cDNA sequences in dbEST database	>20,000
Number of mutants characterized	>1,000
Mutant genes with linkage data available	>500
Mutant genes placed on genetic map	370
Molecular markers on recombinant inbred map	460
Papers published on *Arabidopsis* (1/94 to 11/95)	1,235
Abstracts published on *Arabidopsis*(1/94 to 11/95)	>500
Participants at 1995 *Arabidopsis* Meeting in Madison	>600
Email subscribers to *Arabidopsis* News group	>870
Seed lines distributed by stock centers (past year)	>50,000
Clones distributed by stock centers (past year)	>9,000
T-DNA tagged lines generated	>25,000

Many of the short-term goals for Arabidopsis research outlined in last year's progress report have been completed. These include:

- The announcement of competitive grant programs to support large-scale genomic sequencing projects in the United States and the European Union.

- The addition of more than 5,000 new Expressed Sequence Tags (ESTs) to existing databases.

- The determination of map locations for more than 300 EST clones.

- Continued research on the structure and function of large numbers of cloned genes involved in almost every aspect of plant growth and development.
- Progress towards the development of technologies needed for large-scale genomic sequencing and the establishment of a new generation of Arabidopsis databases.

Recent advances in basic research made with Arabidopsis by researchers world-wide are presented in some detail in this report. Selected references to original research papers, reviews, and meeting reports are included to help the reader obtain additional information on the current status of Arabidopsis research. Recent scientific advances include:

- Isolation and characterization of genes involved in fundamental biological processes such as:
    - Flowering, embryogenesis, and vegetative development
    - Plant responses to light, hormones, and pathogen attack
    - Plant responses to abiotic environmental stresses
    - Plant biochemistry, cell biology, and circadian rhythms

- Publication of a detailed physical map of chromosome 4 by Renata Schmidt, Caroline Dean, and colleagues at the John Innes Centre, Norwich, UK.
- Successful utilization of heterologous (maize) transposons for tagging linked genes, isolating regulatory sequences, and producing chromosomal rearrangements.
- Demonstration that the ETR1 protein of Arabidopsis is an ethylene receptor, the first unequivocal demonstration of hormone receptor function in any plant.
- Successful transfer of genes regulating flower development in Arabidopsis to plants relevant to agriculture and forestry, a major breakthrough that was accompanied by considerable media attention.
- Rapid growth of information on Arabidopsis available through the World Wide Web.

In addition to presenting an overview of scientific progress, technological advances, and community activities, this report contains detailed appendices with information on:

- Email addresses, WWW sites, and selected publications where additional information on Arabidopsis research can be obtained.

- The number of publications on Arabidopsis (1/94 to 11/95) arranged by journal.

- A list of 500 mutant genes for which recombination data have been obtained, along with a companion list of reference laboratories and email addresses.
- An updated classical map of 370 mutant genes of Arabidopsis and a recombinant inbred map that contains 460 molecular markers.

- The physical map of chromosome 4 published by Renata Schmidt and colleagues.

Goals for the Future

The major goal for 1996 is to begin the next phase of the multinational effort to sequence the entire nuclear genome of *Arabidopsis*. This involves the establishment and initial funding of a limited number of collaborative research groups that are qualified and equipped to generate large-scale genomic sequence for

immediate use by the *Arabidopsis* community. Additional goals for the coming year include:

- Expansion and integration of physical, molecular, and genetic maps of *Arabidopsis*.
- Continued advances in the isolation and characterization of informative mutants and essential genes.
- Development of technologies that can readily test the functional significance of genomic sequences identified during the course of this project.
- Continued support for the stock centers and associated databases that provide a vital service to the *Arabidopsis* community.
- Increased application of the information and resources gained through basic research with *Arabidopsis* to fundamental questions in plant biology.

DEVELOPMENT OF COMMUNITY RESOURCES

Significant advances continue to be reported in the isolation and characterization of informative mutants, the construction of genetic and physical maps, the sequencing of selected genomic regions and large numbers of cDNAs, the maintenance of biological resource centers, the development of informatics and communication networks, and the sharing of research information at workshops and conferences. Examples of recent progress in these areas of common interest to the *Arabidopsis* community are presented here.

Identification and Mapping of Mutations

One of the original goals of the *Arabidopsis* Genome Project was to saturate for mutations using both chemical and insertional mutagenesis. Significant advances have been made in this area since the initial plan was outlined in 1990. The continued identification of mutants with novel phenotypes indicates that saturation is still far from complete, but the frequent discovery of allelism between mutants isolated in different laboratories suggests that many of the target genes have already been identified. It has therefore become essential to map the locations of mutant genes and perform allelism tests with known mutants that map to the same chromosomal region before publishing descriptions of recently-identified mutants. In order to facilitate this process, a list of 500 mutant genes of *Arabidopsis* for which recombination data have been obtained is presented in Appendix 3 of this report. Regular updates of this list will be made available through the World Wide Web. At least 500 additional mutants have been isolated and characterized to some extent but not yet assigned to a linkage group. One goal for the coming year will be to continue mapping these mutations in order to establish a more comprehensive list of genes identified by mutation. The current genetic map of *Arabidopsis* includes over 370 genes identified by mutation. A text version of this map is presented in Appendix 4. The following table illustrates the current status of "classical" mapping efforts in *Arabidopsis.* Examples of recent advances made with some of these mutants are presented elsewhere in this report.

Overview of Linkage Information for Genes Identified by Mutation

Chromosome	Genes on Map	Genes Assigned to Chromosome	Total
1	102	29	131
2	50	19	69

3	63	25	88
4	66	20	86
5	92	34	126
Total	373	127	500

The molecular cloning of more than 30 genes identified by mutation was reported at the *Arabidopsis* Meeting in June. Among these are AUX1, AXR4/RGL1, CER2, CER3, CER4, CRC, EMB30/GNOM, ERA1, ERS, FAH1, FCA, GAI, HLS1, KN, PRL, RCN1, SCR, SEX1, SPY, SUS2, TFL1, TIR1, TT12, UFO, and ZWI. The production and analysis of large populations of T-DNA insertional mutants continued over the past year. These efforts should play an important role in gene isolation for several years to come. The current focus of the French effort headed by Michel Caboche and George Pelletier (INRA, Versailles) is to make T3 seeds of more than 10,000 T-DNA lines available for distribution through the Nottingham Stock Centre. Advances were also reported in the efficient isolation and mapping of T-DNA insert junctions by Robert Whittier (Tsukuba, Japan) and in the recovery of T-DNA insertions in known genes by Richard Meagher (University of Georgia) and Ken Feldmann (University of Arizona).

Transposon tagging continued to emerge as a powerful tool for gene identification in *Arabidopsis*. Although the first reports of tagging *Arabidopsis* genes with maize transposons were presented several years ago, a number of significant advances were reported over the past year. The value of large-scale experiments involving gene and promoter trapping was clearly demonstrated, the first example of targeted tagging with a linked transposon was reported, the utility of the En/Spm system for gene tagging in *Arabidopsis* was documented, the nature and movement of retrotransposons was examined in more detail, and the Cre-lox system was incorporated into a Ds-based system for studies of insertional mutagenesis and chromosomal rearrangements in *Arabidopsis*. Additional information on recent advances in gene mapping, trapping, and tagging can be obtained from the following representative publications.

Aarts MGM, Corzaan P, Stiekema WJ, Pereira A (1995) A 2-element enhancer-inhibitor transposon system in *Arabidopsis* thaliana. Mol. Gen. Genet. 247: 555-564.

Altmann T, Felix G, Jessop A, Kauschman A, Uwer U, Penacortes H, Willmitzer L (1995) Ac/Ds transposon mutagenesis in *Arabidopsis* thaliana: Mutant spectrum and frequency of Ds insertion mutants. Mol. Gen. Genet. 247: 646-652.

Franzmann LH, Yoon ES, Meinke DW (1995) Saturating the genetic map of *Arabidopsis* thaliana with embryonic mutations. Plant J. 7: 341-350.

James DW, Lim E, Keller J, Plooy I, Ralston E, Dooner HK (1995) Directed tagging of the *Arabidopsis* FATTY ACID ELONGATION-1 (FAE1) gene with the maize transposon activator. Plant Cell 7: 309-319.

Liu G-Y, Mitsukawa N, Oosumi T, Whittier RF (1995) Efficient isolation and mapping of *Arabidopsis* thaliana T-DNA insert junctions by thermal asymmetric interlaced PCR. Plant J. 8: 457-463.

Lucas H, Feuerbach F, Kunert K, Grandbastien MA, Caboche M (1995) RNA-mediated transposition of the tobacco retrotransposon TNT1 in *Arabidopsis* thaliana. EMBO J 14: 2364-2373.

McKinney EC, Ali N, Traut A, Feldmann KA, Belostotsky DA, McDowell JM, Meagher RB (1995) Sequence-based identification of T-DNA insertion mutations in *Arabidopsis*: actin mutants act2-1 and act4-1. Plant J. 8: 613-622.

Osborne BI, Wirtz U, Baker B (1995) A system for insertional mutagenesis and chromosomal rearrangement using the Ds transposon and Cre-lox. Plant J. 7: 687-701.

Smith DL, Fedoroff NV (1995) A gene expressed in lateral and adventitious root primordia of *Arabidopsis*.

Plant Cell 7: 735-745.

Springer PS, McCombie WR, Sundaresan V, Martienssen RA (1995) Gene trap tagging of PROLIFERA, an essential MCM2-3-5 like gene in *Arabidopsis*. Science 268: 877-880.

Sundaresan V, Springer P, Volpe T, Haward S, Jones JDG, Dean C, Ma H, Martienssen R (1995) Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. Genes Dev. 9: 1797-1810.

Physical Mapping and Genome Analysis

Several major advances in physical mapping and genome analysis were reported over the past year. Perhaps the most dramatic was the publication of a nearly complete physical map of chromosome 4 by Renata Schmidt, Caroline Dean, and colleagues at the John Innes Centre (UK). A copy of this map is presented in Appendix 7. Molecular characterization of the *Arabidopsis* genome also continued to benefit from a number of related efforts, including the addition of new molecular markers to the recombinant inbred map (Appendix 6), continued analysis of the large-insert YAC library developed through a collaborative effort in France, and development of a P1 genomic library by Robert Whittier in Japan and a BAC library by Rod Wing in the United States. The current status of the YAC contig map is as follows: chromosome 1 (42 contigs, 65% coverage), chromosome 2 (4 contigs, 80% coverage), chromosome 3 (42 contigs, 60% coverage), chromosome 4 (4 contigs, over 90% coverage), and chromosome 5 (35 contigs, 85% coverage). Many laboratories have contributed to this effort, including those of Joe Ecker (University of Pennsylvania), Howard Goodman (Massachusetts General Hospital), and Caroline Dean (John Innes Centre). The current status of genome analysis in *Arabidopsis* has recently been reviewed by Goodman, Ecker, and Dean.

The European Union Genome Sequencing Project is presently in its final year. Progress was initially slow as participants learned new methods and trained their teams, but production is now increasing at a steady rate. To date over 850 Kb of genomic sequence has been deposited in databases, and the eight laboratories working on the *FCA* region on chromosome 4 have accumulated over 650 Kb of sequence. There are still several gaps in the sequence, but it is anticipated that a 1 Mb contiguous region will be completed by February, 1996. Over 1.2 Mb of contiguous cosmids have been distributed to participating laboratories, and by the end of 1996 it is hoped that the sequence of this region will extend to 1.8 Mb. This genomic region is rich in putative genes, with an average density of one gene in every 5.5 Kb, about the same as in *C. elegans*. Putative genes are identified with some certainty by genefinder programs. The XGRAIL program has been remarkably effective in predicting complex exonintron junctions. Of the 180 putative genes identified, only 4% had been previously sequenced, 20% have no known homologs, and 75% are similar to genes previously identified in other organisms. Of this latter group, half are similar to other plant genes and half represent genes not previously identified in plants. EST matches (rice and *Arabidopsis*) were found for 25% of the putative genes, and 35% of these EST matches were multiple hits, presumably to highly expressed genes. A systematic analysis of cognate cDNAs over some of the *FCA* region is being carried out to assess the efficacy of the genefinder programs and to provide more information for future programs. Putative genes identified to date include those coding for dehydrogenases, membrane transport proteins, terpene alkaloid biosynthetic enzymes, steroid binding proteins, and viral attachment membrane proteins. The goal of this initial project is to identify 400 novel genes. A proposal is currently being prepared to sequence the rest of chromosome 4 (about 3.5 Mb of the top arm and the remaining 11.5 Mb of the bottom arm) over the next three years. A small network of experienced sequencers, each with a relatively high capacity, has been assembled for this project. Genomic sequencing of large chromosomal regions is set to begin in the United States as well, following an interagency (DOE/NSF/USDA) request for proposals to establish a limited number of sequencing centers for the *Arabidopsis* Genome Project. The combined target output for these centers is 10 Mb of genomic sequence within 3 years. The long-term goal is to complete the entire genome

over the next 8 years. The application deadline for the initial phase is January, 1996, with large-scale sequencing scheduled to begin later in the year. Details are presented in NSF publication 95-159. Many laboratories have already contributed a considerable amount of sequence information for specific genes. A recent version of GenBank (release 91.0, 10/15/95) includes 22,356 entries on nuclear genes of *Arabidopsis* totaling more than 10.6 Mb of DNA. Although some of this sequence is clearly redundant, only four organisms have more sequence information in the database: humans, the nematode, yeast, and mice. Additional information on recent genome studies in *Arabidopsis* can be obtained from the following representative publications.

Goodman HM, Ecker JR, Dean C (1995) The genome of *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA 93: 10831-10835.

Liu Y-G, Mitsukawa N, Vazquez-Tello A, Whittier RF (1995) Generation of a high-quality P1 library of *Arabidopsis* suitable for chromosome walking. Plant J. 7: 351-358.

Schmidt R, West J, Love K, Lenehan Z, Lister C, Thompson H, Bouchez D, Dean C (1995) Physical map and organization of *Arabidopsis thaliana* chromosome 4. Science 270: 480-483.

Zhang H-B, Zhao X, Ding X, Paterson AH, Wing RA (1995) Preparation of megabase-size DNA from plant nuclei. Plant J. 7: 175-184.

cDNA Sequencing Projects: The goal of the cDNA sequencing program is to identify the estimated 20,000 expressed genes in *Arabidopsis*. Two major groups have contributed to this effort, one located at Michigan State University, headed by Thomas Newman and funded by NSF and DOE, and the other a consortium of laboratories in France supported initially by the French government and continued within the framework of the ESSA (European Scientists Sequencing *Arabidopsis*) project funded by the European Commission. Combined, these two groups have identified approximately 14,000 non-redundant expressed sequence tags (ESTs) and deposited over 22,400 ESTs in the dbEST database maintained by the National Center for Biotechnology Information (NCBI). This suggests that over 70% of the expressed genes in *Arabidopsis* have already been identified. BLASTX, BLASTA and FASTA analyses indicate probable function or identity for approximately 40% of the unique sequences. The MSU project has averaged almost 1,000 EST submissions per month for deposition to NCBI over the past year, including presumed redundant sequences. Emphasis in the French program has recently been placed on obtaining sequences from less abundant cDNAs and sequencing both 5' and 3' ends of non-redundant clones. Continued progress was also reported in the laboratory of Marc Van Montagu in Belgium towards the establishment of a protein database of *Arabidopsis* that may provide complementary information on patterns of gene expression.

cDNA Libraries: Nine French laboratories located throughout the country have collaborated on sequencing clones from ten different lambdaZAP libraries from developing siliques, flower buds, etiolated seedlings, cell suspension cultures at different stages, cultured leaf strips, green shoots, flowering tips, dry seeds, and cell suspensions inoculated with the bacterial pathogen, *Xanthomonas campestris* pv *campestris*. The MSU project has utilized the Lambda PRL2 cDNA library derived from equal quantities of four pools of mRNA from etiolated seedlings, roots grown in culture, rosette leaves, and reproductive structures. The vector is BRL's lambda ZipLox. Subtraction libraries derived from the lambda PRL2 library are now being used to obtain rare cDNAs. Additional libraries from pollen and ethylene-treated seedlings are under construction.

Informatics: Sequences generated by the French program are sent to a central database in Toulouse for analysis, where redundancies are identified using FASTA, transferred to the EMBL/*Arabidopsis* databank in Martinsried, Germany, where payment is made for unique sequences, and later submitted to NCBI dbEST (accessible via a WWW site at http://www.ncbi.nlm.nih.gov/dbEST/index.html). The Plant Molecular Informatics Center at the University of Minnesota handles the analysis and distribution of MSU sequence data. This informatics group also provides a WWW site (http://www.cbc.med.umn.edu) for key word and motif searching and a considerable amount of information on desired clones. This data set includes ESTs from both the French and MSU programs, along with sequences from rice, maize, loblolly pine and *Brassica*

napus. At present, over 32,000 plant ESTs are available on the server. A plant DNA database query system with tutorial is also provided for additional access to sequence information.

Distribution of cDNA clones: ESTs from both the MSU and French projects are distributed by the ABRC at Ohio State University. Excluding batch requests from groups involved in genome mapping efforts, over 3,600 clones from the MSU project were requested through September, 1995. The present rate of requests is approximately 200 clones per month for the MSU and French groups combined. The following table indicates the approximate distribution of sequences to NCBI. Publications listed below contain additional details.

SUBMITTED EST SEQUENCES	MSU	French	Total
Unique Sequences	9000	5200	14200
5'/3' of Same Clone	200	2000	2200
Total Submitted to NCBI	17200	5200	22400

Cooke R et al. (1996) Further progress towards a catalogue of all *Arabidopsis* genes: analysis of a set of 5000 non-redundant ESTs. Plant J. 9: 101-124.

Newman T et al. (1994) Genes galore: a summary of the methods for accessing the results of large scale partial sequencing of anonymous *Arabidopsis* thaliana cDNA clones. Plant Physiol. 106: 1241-1255.

Biological Resource Centers

The Nottingham Arabidopsis Stock Centre (NASC) at the University of Nottingham (UK) and the Arabidopsis Biological Resource Center (ABRC) at Ohio State University (USA) continue to serve the needs of *Arabidopsis* researchers worldwide. Over 50,000 samples of seeds, 8,000 individual clones, and many sets of hybridization filters from large-insert genomic libraries were shipped by these centers in the past year alone. In addition, significant numbers of seed and DNA stocks were added to existing collections. Among the ABRC seed stocks acquired during the past year were many unique mutant lines. Approximately 100 of these are included in the 1995 catalog and another 70 should be available soon. Other recent additions include 1,600 new TDNA tagged lines, 100 transposon tagged lines, a number of promoter trap lines, and 200 recombinant inbred lines. In addition to the extensive Redei collection, most of the 300 ecotypes and 100 form mutants of the Kranz (AIS) collection are now available. Notable donations to NASC in the last year include 1,000 promoter trap lines from the Dutch Company MOGEN and 1,400 T-DNA lines from George Pelletier at INRA Versailles. All of these materials will be made available early next year. Efforts are also underway to transfer to NASC many additional T-DNA lines generated by the joint French effort.

Several valuable libraries have been added to the DNA collections at ABRC over the past year. The Center now holds a P1 library, a bacterial artificial chromosome (BAC) library, and five YAC libraries in addition to a number of cDNA, cosmid, and genomic libraries. These materials are vital resources for cloning and physical genome efforts in *Arabidopsis*. Distribution of these libraries to researchers has been streamlined by sending blotted filters for initial screening before individual clones are supplied. This should facilitate efforts of many investigators to isolate genes through map-based cloning. The collection of cloned genes available through ABRC has surpassed 100 and is expanding at a rapid pace, as are collections of RFLP stocks. Several thousand new ESTs have been received from Michigan State University, increasing the number of clones from this project to 15,000. In addition, ABRC maintains 3,000 ESTs generated by the French project. Many of the essential genes in *Arabidopsis* should be represented in this combined collection.

To simplify stock ordering, the AIMS web server has been developed in cooperation with Sakti Pramanik and Jin Kim of Michigan State University to serve both ABRC and NASC. This system, which operates from the Web address, http://www.cbc.med.umn.edu, is now utilized for most ABRC orders. The server also has full searching capability for stock and genetic information. Stock images are fully accessible from the Web server and are linked directly to the associated stocks. NASC stock information continues to be developed through its on-line catalogue (URL=http://nasc.nott.ac.uk). A major addition has been the incorporation of 600 color images of stocks. Orders can be placed online, directly from within the catalogue. NASC information is also carried in AAtDB, the AAtDB research companion, and AIMS.

Contacts for Orders and Information:

Randy Scholl, Director

Arabidopsis Biological Resource Center

Mail: Ohio State University, 1735 Neil Avenue, Columbus, OH 43210, USA.

Telephone: 6142929371

FAX: 6142920603

Email: Arabidopsis+@osu.edu

WWW: http://www.cbc.med.umn.edu

Mary Anderson, Director

Nottingham Arabidopsis Stock Centre

Mail: Department of Life Sciences, University of Nottingham, Nottingham, NG7 2RD, UK

Telephone: +44-115-979-1216

FAX: +44-115-951-3251

Email: Arabidopsis@nottingham.ac.uk

WWW: http://nasc.nott.ac.uk

Informatics and Communication

***Arabidopsis* Information Management System (AIMS)**

This database was developed by Sakti Pramanik and associates to provide information for the *Arabidopsis* community and to facilitate ordering from stock centers. ABRC personnel at Ohio State University maintain the AIMS database. The fullfeature version of AIMS was introduced in 1993 and a WWW server was recently added at: . Development of this Web interface has allowed the entire database to be readily accessed. Most orders to ABRC are placed through this system. Many remote sites access AIMS daily, with each conducting an average of 10 database transactions. Data currently stored in AIMS include:

- Information on ABRC and NASC seed stocks, including color pictures of most plant phenotypes.
- Information on *Arabidopsis* clones, ESTs, RFLP and RAPD stocks, and YAC libraries.
- Images of gel band patterns for all RFLP stocks, along with molecular weight standards, and many comparative hybridization results.
- Sequence homology search results for all ESTs, along with full graphical display of sequences and sequence homology, including dynamic links to GenBank, dbEST and the *Arabidopsis thaliana* sequence analysis database.
- Approximately 4,000 references on *Arabidopsis*, including rapid updates of recent publications and all *Arabidopsis* Information Service articles.
- Contact information for 2,700 individuals on the ABRC mailing list.

The AIMS XWindow server can also be used to compare genetic maps and display contig data. Images of stocks are fully accessible from the Web server. The intent is to utilize these images to portray important features of each stock. Images for a selected group of stocks can be compared on the same Web page. For additional information, contact Sakti Pramanik or Randy Scholl at (aimsmanager@aims.msu.edu). General questions may be directed to the ABRC staff at the addresses given in the Biological Resource Centers section of this report.

***Arabidopsis thaliana* Database (AAtDB/AtDB)** This database was developed several years ago to provide the *Arabidopsis* community with a wide range of information in graphic, tabular, and text formats. The most recent release included information on:

- A variety of genetic markers (RFLP, RAPD, classical) and maps (RI, integrated, classical), along with primary recombination data.
- Stock information and phenotypic descriptions of mutants.
- Large numbers of *Arabidopsis* DNA sequences from GenBank, including recent sequence comparisons.
- Contact information for *Arabidopsis* researchers and bibliographic information from Agricola and Medline
- Scanned images of RFLP autoradiograms, photographs of mutant plants, and restriction enzyme digests of RFLP probes

The database was recently moved from Massachusetts General Hospital, where it was maintained by John Morris and Howard Goodman, to Stanford University, where it will be curated and upgraded into the next generation of *Arabidopsis* databases (AtDB) by David Flanders and Mike Cherry. The database is now accessible through the WWW at: http://genome-www.stanford.edu. The Stanford project will extend AAtDB and make information readily available through the WWW. Enhancements include direct access to the *Arabidopsis* database, DNA and protein sequence searches via BLAST and FASTA, and a variety of cross-

links with WWW resources worldwide. The long-term goal of the AtDB project is a new environment designed for the WWW to provide effective access to all relevant genomic and reference information. For additional information, contact Mike Cherry (cherry@genome.stanford.edu) or David Flanders (flanders@genome.stanford.edu). Phone: +1-415-725-3062; FAX: +1-415-723-7016.

BIOSCI/BIONET Networks

The BIOSCI *Arabidopsis* Genome Electronic Conference was established several years ago as part of the BIOSCI/BIONET network. The *Arabidopsis* group has emerged as a model for widespread information exchange worldwide. Access was originally made through USENET or email. This has recently expanded to include WWW access through: http://www.bio.net. By selecting the "Access the BIOSCI/BIONET Newsgroups" hyperlink, individuals can utilize the USENET network without requiring a local news server. Subscriptions from Europe, Africa, and Central Asia can still be addressed via email to:biosci@daresbury.ac.uk; those from the Americas and the Pacific Rim should be addressed to biosci@net.bio.net. Messages to the newsgroup should be addressed to: arabidopsis@net.bio.net. Difficulties with the system should be reported to Dave Kristofferson (BIOSCI/BIONET Manager) at: biosci-help@net.bio.net. Currently there are more than 640 email subscribers on the US mailing list and 230 on the UK list, plus an unknown number of readers on USENET. The addition of WWW links should increase even further the number of individuals with quick access to this important medium for information exchange.

Weeds World and Other Internet Resources

A considerable amount of information on *Arabidopsis* can now be obtained through the World Wide Web. Several examples of relevant servers and home pages are presented in Appendix 1. An electronic *Arabidopsis* newsletter (Weeds World) was also initiated last year to facilitate distribution of information and published reports on *Arabidopsis*. The first several issues have included meeting reports, research papers, and technological advances. Access is through: http://nasc.nott.ac.uk:8300 (Nottingham) or http://weeds.mgh.harvard.edu/ww/home.html (Boston).

Meetings and Workshops

It has become impractical to summarize every meeting and workshop that included a discussion of *Arabidopsis* research because the number of such meetings continues to expand. Most of the recent national and international meetings on plant molecular biology, biochemistry, physiology, development, pathology, and cell biology included presentations on research with *Arabidopsis*. A number of regional meetings and workshops also dealt with *Arabidopsis* techniques. Two notable examples are highlighted in this report: The Sixth International Conference on *Arabidopsis* Research; and The Cold Spring Harbor Course on *Arabidopsis* Molecular Genetics.

Arabidopsis Meeting at Madison

The Sixth International Conference on *Arabidopsis* Research was held June 7-11, 1995 at the Memorial Union on the campus of the University of Wisconsin at Madison. There were 640 registered participants from over 15 different countries, including Australia, Belgium, Canada, Denmark, Finland, France, Germany, Japan, the Netherlands, New Zealand, Korea, Spain, Sweden, Switzerland, Taiwan, the United Kingdom, and the United States. Two thirds of the participants (440/640) presented talks or posters. Abstracts were printed in a book that was distributed to all participants. The meeting included sessions on embryogenesis, seed development, root and shoot development, flowering, environmental effects on development, cell biology, biochemical genetics, growth regulators, and response to pathogens and environmental stress. In keeping with past meetings, all sessions were consecutive rather than concurrent. A

number of workshops on specialized topics also attracted wide participation. Information on scientific advances presented at this meeting can be obtained from detailed reports published in The Plant Cell (Volume 7, pp 1737-1748, 1995) and Weeds World (Electronic *Arabidopsis* Journal available through WWW). Dates and locations for future *Arabidopsis* meetings were also established:

1996 Meeting: June 23-27, 1996

University of East Anglia, Norwich, United Kingdom

Contact: Caroline Dean (Arabidopsis@bbsrc.ac.uk)

1997 Meeting: June 26-30, 1997

University of Wisconsin, Madison, USA

Contact: Rick Amasino (amasino@biochem.wisc.edu)

Cold Spring Harbor Course

The Cold Spring Harbor Course on *Arabidopsis* Molecular Genetics was held July 3-23, 1995 and attracted 16 students from six different countries. The demand for this course was reflected in the large number (approximately 40) of applications received. Student participants included graduate students, postdoctoral fellows, faculty, and government/industry employees. The course was supported by a NSF award. Organizers were XingWang Deng (Yale University), Robert Last (Boyce Thompson Institute), and Daphne Preuss (University of Chicago). The course provided an intensive overview of topics in plant growth and development, focusing on molecular genetic approaches to understanding plant biology. The course included detailed lectures, extensive laboratory experiments, and informal discussions.

Instructors provided an overview of their discipline and specific details on their own work. Discussion topics included plant anatomy; plant development (including flowers, roots, meristems, embryos, and epidermis); perception of light and photomorphogenesis; responses to pathogens and to DNA damage; synthesis and function of hormones and secondary metabolites; nitrogen assimilation; unique aspects of plant cell biology (including the cytoskeleton, cell wall, and chloroplasts); the importance of transposons and *Agrobacterium* for manipulating plant genomes, and current approaches to genome analysis. Instructors included: J. Bender, A. Britt, C. Chapple, J. Chory, X.W. Deng, G. Drews, J. Ecker, S.Y. He, R. Last, A. Lloyd, H. Ma, R. Martienssen, J. Medford, T. MitchellOlds, D. Preuss, P. Quail, J. Schiefelbein, R. Scholl, B. Staskawicz, D. Stern, V. Sundaresan, I. Sussex, T. Voelker, V. Walbot, and P. Zambryski.

The laboratory sessions provided an introduction to important techniques currently used in *Arabidopsis* research. These included studies of *Arabidopsis* development, mutant analysis, in situ detection of RNA, histochemical staining and immunolabeling of proteins, transformation with *Agrobacterium*, transient gene expression, expression of plant proteins in microorganisms, detection and analysis of plant pathogens, genetic and physical mapping, and gene cloning.

OVERVIEW OF SCIENTIFIC ADVANCES

In addition to the expanding resources described above, dramatic progress continued to be made in characterizing specific genes and establishing a solid foundation of basic knowledge in plant biology. Some of these advances are highlighted in this section of the report, along with selected references for additional information. The Steering Committee asks members of the community to realize that it was impractical to describe every significant achievement here, and that selected examples were chosen to illustrate the breadth of modern research with *Arabidopsis*.

Flower Development

The study of flower development is continuing to provide new, and often surprising results. Last year's highlight was the finding that either of two *Arabidopsis* genes, *LEAFY* and *APETALA1*, is sufficient to turn shoots into flowers. It had been known for a while that these genes are required for the proper formation of flowers, as plants without functional copies of *LEAFY* and *APETALA1* produce extra shoots in place of flowers. Both genes had been cloned several years ago, and found to be expressed only in flowers, but not in vegetative structures, consistent with a specific function of these genes in flower development. However, it was still unclear what the potential of each gene alone was. To answer this question, two groups generated transgenic *Arabidopsis* plants in which either *LEAFY* or *APETALA1* was constitutively expressed (Mandel and Yanofsky, 1995; Weigel and Nilsson, 1995).

The results were dramatic: lateral shoots in transgenic plants are converted into solitary flowers. Furthermore, the main shoot is eventually also consumed in the formation of a terminal flower. The flowers look normal and are fertile, demonstrating that either gene can switch on all other genes required for normal flower development. An important consequence of the shoot-to-flower conversion is that the transgenic plants flower earlier than normal plants. Encouraged by these results, Weigel and Nilsson (1995) went on to test the effects of the *LEAFY* gene in other plants, among them aspen. This tree is of considerable economic importance in Europe, where it is a major source for pulp, and the same is true for the closely related cottonwood in the US. Aspen trees normally flower only after one or two decades. In contrast, transgenic aspens that constitutively express *LEAFY* flower within a few months. This observation illustrates how results obtained with *Arabidopsis* are often applicable to very different species. One immediate practical application of this research is that flowering time and thus generation time can be reduced, which should prove useful to breeders.

Significant progress had also been made in understanding upstream acting genes that regulate when *LEAFY* and *APETALA1* are switched on during the life cycle. *Arabidopsis* plants flower much faster under conditions of long days and short nights than under conditions of short days and long nights. A key element in the signal transduction pathway regulated by day length is the *CONSTANS* gene, which is required for early flowering under long days. This gene has now been isolated and found to be expressed at high levels in long days, but only very low levels in short days (Putterill et al., 1995). Experiments with transgenic plants already show that *CONSTANS* levels are indeed causally related to flowering time. Plants with increased levels of *CONSTANS* gene product (from multiple copies of the wild-type gene) flower earlier than normal plants. Thus, manipulation of the *CONSTANS* gene should provide yet another way to alter flowering time.Finally, the cloning of the *SUPERMAN* (*FLO10*) gene has been an important step forward in understanding how plant cells communicate with each other (Sakai et al., 1995). The gene had previously been identified through mutations that cause additional stamens to be produced at the expense of carpels. Originally, it was thought that this was related to defects in repressing the transcription of the homeotic genes that promote stamen development. However, it turns out that *SUPERMAN* functions downstream of the homeotic genes, and has two related functions. It limits proliferation of the cells where it is expressed, and at the same time it prevents a negative signal that tells neighboring cells to stop dividing. Thus, when

SUPERMAN is inactive, the stamen precursors proliferate too much and at the same time repress proliferation of the adjacent carpel precursors. The next step will be to elucidate the molecular nature of the signal that is negatively regulated by *SUPERMAN*.

Mandel MA, Yanofsky MF (1995) A gene triggering flower development in *Arabidopsis*. Nature *377*: 522-524.

Putterill J, Robson F, Lee K, Simon R, Coupland G (1995) The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. Cell *80*: 847-857.

Sakai H, Medrano LJ, Meyerowitz EM (1995) Role of *SUPERMAN* in maintaining *Arabidopsis* floral whorl boundaries. Nature 378: 199-203.

Weigel D, Nilsson O (1995) A developmental switch sufficient for flower initiation in diverse plants. Nature *377*: 495-500.

Embryo Development

Many laboratories have contributed to the study of embryogenesis in *Arabidopsis* (see Goldberg et al., 1995; Jurgens, 1995; Meinke, 1995). Significant advances were reported this past year in our understanding of the origin of root and shoot apical meristems and the chromosomal locations and molecular functions of genes expressed during embryo development. Mutants defective in embryogenesis continued to play an important role in uncovering interesting genes. In a striking example of how research with *Arabidopsis* and maize can be combined to solve a complex puzzle, the recessive *shootmeristemless* mutant of *Arabidopsis*, which fails to form a shoot apex during embryogenesis, was shown to be defective in a homolog of the *Knotted* gene of maize, which encodes a homeodomain protein that causes inappropriate cell divisions when ectopically expressed in leaves (Long et al., 1996). Research with *Arabidopsis* in this case provided a missing piece to the puzzle because only dominant gain-of-function *Knotted* mutants had been described in maize, and consequently the normal function of this gene throughout development was uncertain. *STM* provides the best example to date of a cloned gene in *Arabidopsis* that plays a direct role in the establishment of an important compartment or pattern in the developing embryo. MADS-box genes were shown to be expressed during embryogenesis as well (Heck et al., 1995; Rounsley et al., 1995) but their function at this stage remains to be determined. The origin of root patterns during embryogenesis was also explored in some detail as described elsewhere in this report.

Significant progress continued to be reported in the mapping and molecular characterization of embryo-defective mutants. Recombination data with visible markers have been obtained for more than 180 *emb* mutants, and 125 different genes have been placed on the genetic map (Franzmann et al., 1995; Appendix 5). If current estimates of 500 target *EMB* genes are correct, then at least 25% of all genes that mutate to give an embryo-defective phenotype have been mapped. The normal functions of some of these genes are gradually being identified. Published reports include *EMB30/GNOM* (Shevell et al., 1994), which encodes a protein with restricted regions of similarity to the Sec7 protein of yeast and may therefore be associated with intracellular transport, and FUS6 (Castle and Meinke, 1994), which encodes a protein with similarity to human proteins involved in G-protein mediated signal transduction pathways (Chamovitz and Deng, 1995). In addition, several laboratories reported on molecular characterization of mutants disrupted in embryogenesis at the *Arabidopsis* Conference in Madison. Wolfgang Lukowitz and Gerd Jurgens (University of Tubingen) reported that the *knolle* mutant, which appears to be defective in radial patterning during embryogenesis, is disrupted in a gene that encodes a protein with similarity to syntaxins, vesicle docking proteins that may play an important role in cytokinesis in plants. Another mutant (*fass/tonneau/emb40*) with normal patterning but abnormal cell and body shape was recently shown to lack a preprophase band and

exhibit relatively normal differentiation despite cytoskeletal defects (Traas et al., 1995). Ryuji Tsugeki and Nina Fedoroff (Carnegie Institute, Baltimore; Pennsylvania State University) reported that another embryo-defective mutant contained a Ds insertion in a gene that encoded a ribosomal protein similar to the nuclear-encoded S16 protein localized in mitochondria of *Neurospora*. One impressive aspect of this work was the speed at which sequence information could be obtained from Ds insertion lines using direct sequencing of TAIL-PCR products. Ursala Uwer and Lothar Willmitzer (Berlin, Germany) described an embryo-defective mutant that appeared to be disrupted in a gene with similarity to a bacterial glycyl tRNAsynthetase. Brian Schwartz and David Meinke (Oklahoma State University) reported that an abnormal suspensor mutant (*sus2*) contains a T-DNA insertion in a large gene with a high degree of sequence similarity to the PRP8 gene of yeast, which functions in RNA splicing. Mutants defective in this splicing factor have not previously been described in any multicellular eukaryote. Genes with important and diverse functions in growth and development are therefore being identified at an expanding rate. Among the many mutants described at Madison, one of the most intriguing phenotypes was that of the fertilization-independent endosperm/seed mutants reported by Abed Chaudhury (CSIRO, Australia) and John Harada for Robert Fischer (University of California), in which seed enlargement and a limited amount of endosperm development occurs in the absence of fertilization. Further analysis of these mutants may provide valuable insights into the regulation of early stages of embryo and endosperm development. A report from Sacco De Vries (Wageningen, the Netherlands) also offered hope that an efficient system of somatic embryogenesis in *Arabidopsis* may soon be established.

Castle LA, Meinke DW (1994) A *FUSCA* gene of *Arabidopsis* encodes a novel protein essential for plant development. Plant Cell 6: 25-41.

Chamovitz DA, Deng X-W (1995) The novel components of the *Arabidopsis* light signaling pathway may define a group of general developmental regulators shared by both animal and plant kingdoms. Cell 82: 353-354.

Franzmann LH, Yoon ES, Meinke DW (1995) Saturating the genetic map of *Arabidopsis thaliana* with embryonic mutations. Plant J. 7: 341-350

Goldberg RB, de Paiva G, Yadegari R (1994) Plant embryogenesis: zygote to seed. Science 266: 605-614.

Heck GR, Perry SE, Nichols KW, Fernandez DE (1995) *AGL15*, a MADS domain protein expressed in developing embryos. Plant Cell 7: 1271-1282.

Jurgens G (1995) Axis formation in plant embryogenesis: cues and clues. Cell 81: 467-470.

Long JA, Moan EI, Medford JI, Barton MK (1996) A member of the *KNOTTED* class of homeodomain proteins encoded by the *SHOOTMERISTEMLESS* gene of *Arabidopsis*. Nature 379: 66-69.

Meinke DW (1995) Molecular genetics of plant embryogenesis. Ann. Rev. Plant Physiol. Plant Mol. Biol. 46: 369-394.

Rounsley SD, Ditta GS, Yanofsky MF (1995) Diverse roles for MADS box genes in *Arabidopsis* development. Plant Cell 7: 1259-1269.

Shevell DE, Leu W-M, Giolmor CS, Xia G, Feldmann KA, Chua N-H (1994) *EMB30* is essential for normal cell division, cell expansion, and cell adhesion in *Arabidopsis* and encodes a protein that has similarity to Sec7. Cell 77: 1051-1062.

Traas J, Bellini C, Nacry P, Kronenberger J, Bouchez D, Caboche M (1995) Normal differentiation patterns in plants lacking microtubular preprophase bands. Nature 375: 676-677.

Reproductive Development

Seedling development normally consists of a vegetative phase, followed by a reproductive phase. Mutations in either of the two *EMBRYONIC FLOWER (EMF)* genes alter this developmental program, causing plants to bypass vegetative development and proceed directly to floral development. The *EMF* genes interact with other genes known to be required for floral meristem or floral organ formation, including *LFY* and *AG* (Yang et al., 1995). Another gene required for floral development is *UFO* (*UNUSUAL FLORAL ORGANS*). Analysis of many *ufo* alleles shows it serves to distinguish flowers from shoots, defining boundaries within floral primordia and controlling cell proliferation during the growth of floral organs (Levin and Meyerowitz, 1995). *UFO* shows significant sequence similarity with the *Antirrhinum* meristem and organ identity *FIM* gene, illustrating the utility of analyzing development in these two model systems (Ingram et al., 1995).

Many genes that regulate the initiation of floral organs have been defined, but more recently attention has turned to genes that control the structure and function of those organs. The pistil and stamens are particularly important, as these organs house the developing gametophytes. New analysis of the *SUPERMAN* (*FLO10*) gene shows it plays a dual role in regulating both stamens and pistils. Mutations in *SUP* (*FLO10*) increase stamen number, decrease the size of the pistil, and also alter ovule structure, resulting in aberrant ovules that are bilaterally symmetrical (Gaiser et al., 1995). In contrast, mutations in the *ETTIN* gene specifically affect development of the pistil, causing an abnormal proliferation of transmitting tract tissue on the pistil surface and alterations in the structure of the style and stigma (Sessions and Zambryski, 1995). *ETTIN* function may be required to mark positions along the longitudinal and transverse axis of the pistil. Within the pistil, the development of ovules and the female gametophyte require the action of many genes. Interestingly, mutations in genes required for ovule development also can affect pollen tube growth through the pistil, supporting the idea that ovules send long-range signals that direct pollen tube growth (Hulskamp et al., 1995). Insertional mutagenesis using a promoterless reporter is proving to be a powerful method for identifying additional genes that function in ovules and gametophytes (Sundaresan et al., 1995). One such gene, *PROLIFERA*, was shown to be present in all dividing cells (particularly in the female gametophytes) and share significant sequence similarity with yeast proteins required for DNA replication (Springer et al., 1995).

Gaiser JC, Robinson-Beers K, Gasser CS (1995) The *Arabidopsis SUPERMAN* gene mediates asymmetric growth of the outer integument of ovules. Plant Cell 7: 333-345.

Hulskamp M, Schneitz K, Pruitt RE (1995) Genetic evidence for a long-range activity that directs pollen tube guidance in *Arabidopsis*. Plant Cell 7: 57-64.

Ingram GC, Goodrich J, Wilkinson MD, Simon R, Haughn RW, Coen ES (1995) Parallels between *UNUSUAL FLORAL ORGANS* and *FIMBRIATA*, genes controlling flower development in *Arabidopsis* and *Antirrhinum*. Plant Cell 7: 1501-1510.

Levin JZ, Meyerowitz EM (1995) *UFO*: An *Arabidopsis* gene involved in both floral meristem and floral organ development. Plant Cell 7: 529-548.

Sessions RA, Zambryski PC (1995) *Arabidopsis* gynoecium structure in the wild and in ettin mutants. Development 121: 1519-1532.

Springer PS, McCombie WR, Sundaresan V, Martienssen RA (1995) Gene trap tagging of *PROLIFERA*, an essential *MCM2-3-5*-like gene in *Arabidopsis*. Science 268: 877-880.

Sundaresan V, Springer P, Volpe T, Haward S, Jones JDG, Dean C, Ma H, Martienssen R (1995) Patterns of gene action in plant development revealed by enhancer trap and gene trap transposable elements. Genes Dev. 9: 1797-1810.

Yang CH, Chen LJ, Sung ZR (1995) Genetic regulation of shoot development in *Arabidopsis*: role of the *EMF* genes. Dev. Biol. 169: 421-435.

Cell Growth and Development

The past year has seen important advances toward understanding *Arabidopsis* genes that control plant development on a cellular level. Some of these genes regulate cell expansion while others specify cell fate. Together these genes contribute to diverse developmental processes, from the formation of a network of roots and root hairs to the elaboration of trichomes that cover the surface of stems and leaves.

The *DIMINUTO* (*DWARF1*) gene controls cell elongation in many plant tissues (Takahashi, et al., 1995). Cells from *dim* (*dwf1*) mutants are small in size, resulting in short hypocotyls, stems, petioles, and roots. A T-DNA insertion in this gene does not affect responses to hormones or light, but it does alter the expression of ß-tubulin, a cytoskeletal protein that plays a key role in the assembly of plant cell walls. The *SABRE* gene affects cell expansion in a slightly different manner. In the root cortex, cells from *sabre* mutants expand in an inappropriate direction (Aeschbacher et al., 1995). Because a reduction in ethylene levels partially rescues the mutant phenotype, *SABRE* may function normally to counter ethylene effects. Additional genes that regulate root cell elongation have been identified (Hauser et al., 1995). Molecular dissection of these genes will help to elucidate the role of cell elongation in plant development.

Specification of cell fate also plays a critical role in plant development. The *TTG* gene is emerging as a central regulator of cell fates in the epidermis. In roots, *TTG* specifies the location of root hair cells (Galway et al., 1994). In stems and leaves, *TTG* initiates trichome formation (Lloyd et al., 1994). The analysis of trichome assembly, which requires more than 21 genes, is emerging as a useful model for plant cell development (Hulskamp et al., 1994).Aeschbacher RA, Hauser MT, Feldmann KA, Benfey PN (1995) The SABRE gene is required for normal cell expansion in *Arabidopsis*. Genes Dev. 9: 330-340.

Galway ME, Masucci JD, Lloyd AM, Walbot V, Davis RW, Schiefelbein JW (1994) The TTG gene is required to specify epidermal cell fate and cell patterning in the *Arabidopsis* root. Dev. Biol. 166: 740-754.

Hauser MT, Morikami A, Benfey PN (1995) Conditional root expansion mutants of *Arabidopsis*. Development 121: 1237-1252.

Hulskamp M, Misera S, Jurgens G (1994) Genetic dissection of trichome cell development in *Arabidopsis*. Cell 76: 555-566.

Lloyd AM, Schena M, Walbot V, Davis RW (1994) Epidermal cell fate determination in *Arabidopsis*: patterns defined by a steroid-inducible regulator. Science 266: 436-439.

Takahashi T, Gasch A, Nishizawa N, Chua N-H (1995) The *DIMINUTO* gene of *Arabidopsis* is involved in regulating cell elongation. Genes Dev. 9: 97-107.

Root Development

The past year saw significant progress in the understanding of root development in *Arabidopsis*. The root meristematic initials have been considered the primary determinants of root radial patterning. A genetic analysis suggested that the radial organization of the root may be regulated, in large part, by the genes that are responsible for the radial organization of the embryo (Scheres et al., 1995). In a related development, it was found using laser ablation that root meristematic initials are dependent on signals from surrounding cells to know when and how to divide (van den Berg et al., 1995). Analysis of mutants also implicated cell signalling in determining the cell fate of the root epidermis (Masucci and Schiefelbein, 1994; Galway et al., 1995). The existence of separate genetic programs for division of embryonic root and of root meristematic initials was suggested by two mutants that make abnormally short roots (Cheng et al., 1995).

The development of lateral roots had largely resisted genetic analysis until this past year. Anatomical and physiological studies indicate that lateral root formation in *Arabidopsis* is a twostage process (Laskowski et al., 1995). That auxin may play a role in both stages was indicated by analysis of mutants that have either an excess or reduced number of lateral roots (Boerjan et al., 1995; Celenza Jr. et al., 1995; Hobbie and Estelle, 1995; Simmons et al., 1995). Transposon tagging has been used to identify a gene that is expressed during the earliest stages of lateral root primordia formation (Smith and Fedoroff, 1995).

The regulation of cell expansion is being studied using the *Arabidopsis* root as a model. Analysis of 21 mutations that result in abnormal root cell expansion indicated that microtubules may not play as essential a role in determining the orientation of expansion as previously thought (Hauser et al., 1995). Similar conclusions were drawn from a study of the effect of microtubule stabilizing and destabilizing agents on *Arabidopsis* roots (Baskin et al., 1994). Expansion in the rapidly dividing meristematic cells and in cells in the root elongation zone appear to be regulated by two distinct genetic programs (Baskin et al. 1995). One of the first genes that regulates plant cell expansion was cloned and appears to act by countering the effect of ethylene on root cell expansion (Aeschbacher et al., 1995).

Aeschbacher RA, Hauser M-T, Feldmann KA, Benfey PN (1995) The *SABRE* gene is required for normal cell expansion in *Arabidopsis*. Genes Dev. 9: 330340.

Baskin TI, Wilson JE, Cork A, Williamson RE (1994). Morphology and microtubule organization in *Arabidopsis* roots exposed to oryzalin or taxol. Plant Cell Physiol. 35: 935942.

Baskin TI, Cork A, Williamson RE, Gorst R (1995) *STUNTED PLANT 1*, a gene required for expansion in rapidly elongating but not in dividing cells and mediating root growth response to applied cytokinin. Plant Physiol. 107: 233243.

Boerjan W, Cervera M-T, Delarue M, Beeckman T, Dewitte W, Bellini C, Caboche M, Van Onckelen H, Van Montagu M, Inze D (1995) *superroot*, a recessive mutation in *Arabidopsis*, confers auxin overproduction. Plant Cell 7: 14051419.

Celenza Jr JL, Grisafi PL, Fink GR (1995) A pathway for lateral root formation in *Arabidopsis thaliana*. Genes Dev. 9: 21312142.

Cheng JC, Seeley KA, Sung ZR (1995). *RML1* and *RML2*, *Arabidopsis* genes required for cell proliferation at the root tip. Plant Physiol. 107: 365376.

Galway ME, Masucci JD, Lloyd AM, Walbot V, Davis RW, Schiefelbein JW (1995) The *TTG* gene is required to specify epidermal cell fate and cell patterning in the *Arabidopsis* root. Dev. Biol. 166: 740754.

Hauser M-T, Morikami A, Benfey PN (1995) Conditional root expansion mutants of *Arabidopsis*. Development 121: 12371252.

Hobbie L, Estelle M (1995) The *axr4* auxinresistant mutants of *Arabidopsis thaliana* define a gene important for root gravitropism and lateral root initiation. Plant J. 7: 211220.

Laskowski MJ, Williams ME, Nusbaum HC, Sussex IM (1995) Formation of lateral root meristems is a twostage process. Development 121: 33033310.

Masucci JD, Schiefelbein JW (1994) The *rhd6* mutation of *Arabidopsis thaliana* alters roothair initiation through an auxin and ethyleneassociated process. Plant Physiol. 106: 13351346.

Scheres B, Di Laurenzio L, Willemsen V, Hauser M-T, Janmaat K, Weisbeek P, Benfey PN (1995) Mutations affecting the radial organization of the *Arabidopsis* root display specific defects throughout the radial axis. Development 121: 5362.

Simmons C, Migliaccio F, Masson P, Caspar T, Soll D (1995) A novel root gravitropism mutant of *Arabidopsis thaliana* exhibiting altered auxin physiology. Physiol. Plant. 93: 790798.

Smith DL, Fedoroff NV (1995) *LRP1*, a gene expressed in lateral and adventitious root primordia of *Arabidopsis*. Plant Cell 7: 735745.

van den Berg C, Willemsen V, Hage W, Weisbeek P, Scheres B (1995). Cell fate in the *Arabidopsis* root meristem determined by directional signalling. Nature 378: 6265.

Hormone Research

During the past year, *Arabidopsis* researchers have made a number of important discoveries in the plant hormone field. Foremost among these discoveries is the recent demonstration that the ETR1 protein is an ethylene receptor (Schaller and Bleecker, 1995). This is a landmark study because it is the first unequivocal demonstration of hormone receptor function in any plant. The *ETR1* gene was originally defined by mutation (Bleecker et al., 1988) and cloned using a map-based approach (Chang et al., 1993). These studies showed that ETR1 is required for ethylene response and is similar in structure to the two component regulatory proteins commonly found in bacteria. Now Schaller and Bleecker (1995) have shown that ETR1 binds ethylene when expressed in yeast cells and a mutation that eliminates ethylene response in plants also prevents ethylene binding in yeast. Hua et al. (1995) have shown that the *ERS* gene, a gene related to *ETR1*, may also function as an ethylene receptor. All of the *etr1* mutations that prevent ethylene response are dominant and clustered near the amino terminus of the protein. When a similar mutation was introduced at the identical position in the *ERS* gene, this mutant gene conferred ethylene resistance suggesting that the ERS protein has the same function in ethylene response as ETR1. These studies have recently led to new insight into the mechanism of ethylene action in plant species other than *Arabidopsis*. Wilkinson et al. (1995) have shown that the *Never-ripe* gene in tomato encodes an ETR1-like protein. The *never-ripe* mutant is insensitive to ethylene and deficient in fruit ripening. Strikingly, the mutation lies in the same region of the protein as the *etr1* mutations in *Arabidopsis*. Clearly *Arabidopsis* provides an excellent model for other plants, including crop species.

The other hormones have not been ignored during the past year. A team of researchers headed by Malcolm Bennett at Warwick University have reported the cloning of the *AUX1* gene using a T-DNA tagged allele. The aux1 mutants have agravitropic roots and are resistant to auxin. Sequence analysis indicates that the AUX1 protein contains a number of transmembrane domains and is probably an integral membrane protein (Bennett, unpublished). Although the biochemical function of the protein remains unknown, these results suggest that it may function in auxin perception or transport. Progress has also been reported in the study of IAA metabolism. Bartel and Fink (1995) reported the cloning of a gene that is involved in hydrolysis of IAA-leucine conjugates. The gene, called *ILR1*, was identified by screening for mutants that are resistant to IAA-leucine. The function of IAA-leucine and other IAA-amino acid conjugates is not clear, but they may function as storage forms of IAA. Analysis of the cloned gene will help to solve this longstanding problem. A gene involved in response to gibberellins (GA) has also been cloned during the last year. The *spindly* mutants were identified by screening for plants that elongate in the presence of the GA biosynthesis inhibitor paclobutrazol (Jacobsen and Olszewski, 1993). Phenotypic characterization indicates that *spy* plants display a constitutive GA response. By using a T-DNA tagged allele of *spy*, Olszewski and coworkers at the University of Minnesota cloned the *SPY* gene and showed that it encodes a novel protein with tetratricopeptide repeats. A search of the database indicates that a similar protein of unknown function is found in *C. elegans*, suggesting that *SPY* is a member of a family of regulatory proteins.

A class of plant growth regulators that is often left out of the pantheon of plant hormones are the brassinosteroids. Recent work from Joanne Chory's lab (Salk Institute) has provided new evidence for the

importance of these compounds. The *det2* mutant displays a de-etiolated phenotype when grown in the dark and a number of photoperiodic responses in light-grown plants (Chory et al., 1991). Chory and co-workers recently cloned the *DET2* gene using a map-based approach. Sequence analysis indicates that the gene encodes a protein with high sequence identity to mammalian steroid reductases and the mutant is rescued by brassinolide, a very active brassinosteroid, suggesting that the protein functions in brassinosteroid biosynthesis. These data suggest that brassinosteroids play a role in light-regulated development throughout the *Arabidopsis* life cycle.

Bartel B, Fink GR (1995) *ILR1*, an amidohydrolase that releases active indole-3-acetic acid from conjugates. Science 268: 1745-1748.

Bleecker AB, Estelle MA, Somerville CR, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science 241: 10861089.

Chang C, Kwok SF, Bleecker AB, Meyerowitz EM (1993) *Arabidopsis* ethylene-response gene *ETR1*: Similarity of product to two-component regulators. Science 262: 539-544.

Chory J, Nagpal P, Peto CA (1991) Phenotypic and genetic analysis of *det2*, a new mutant that affects light-regulated seedling development in *Arabidopsis*. Plant Cell 3: 445-460.

Hua J, Chang C, Sun Q, Meyerowitz E (1995) Ethylene insensitivity conferred by *Arabidopsis ERS* gene. Science 269: 1712-1714.

Jacobsen SE, Olszewski NE (1993) Mutations at the *SPINDLY* locus of *Arabidopsis* alter gibberellin signal transduction. Plant Cell 5: 887-896.

Schaller GE, Bleecker AB (1995) High-affinity binding sites for ethylene are generated in yeast expressing the *Arabidopsis ETR1* gene. Science 270: 1809-1811.

Wilkinson JQ, Lanahan MB, Yen H-C, Giovannoni J, Klee HJ (1995) An ethylene-inducible component of signal transduction encoded by *Never-ripe*. Science 270: 1807-1809.

Circadian Rhythms

In higher plants, sunlight serves not only as a source of energy, but also as a developmental signal and timing cue. As daylength varies throughout the year, there is a strong adaptive advantage for plants to be able to anticipate oncoming dawn or dusk at any given time of the year, rather than merely responding to the presence or absence of light. It is the internal circadian clock in plants that allows the organism to prepare its metabolism and developmental strategies in advance of dawn or dusk. A vast array of processes in plants are regulated in a circadian manner, from the opening and closing of stomata, to the control of almost all major metabolic pathways and the induction of flowering in photoperiodism. The understanding of how the circadian clock runs, how it is reset each day by light, and how it regulates such a diverse range of biological functions will have broad significance for plant biology. In the past year, *Arabidopsis* has played a critical role in advancing our knowledge of circadian function. Researchers at the NSF Center for Biological Timing at the University of Virginia have developed novel approaches to measure circadian rhythms.

The promoter of a circadian-regulated photosynthetic gene, *CAB*, was fused to the coding sequence of firefly luciferase. This construct was transferred to *Arabidopsis*, resulting in a population of transgenic plants that exhibited rhythmic bioluminescence. Using ultra-low light video cameras, the rhythmic luminescence was used as a phenotype to screen for mutants with aberrant circadian cycling (Millar et al., 1995a). From a

preliminary screen of 10,000 M2 plants, 21 lines were identified as *toc* (timing of *cab*) mutants. One of these lines, *toc1*, has been shown to encode a central component of circadian regulation in *Arabidopsis*. The *toc1* mutant has a shortened circadian period of 21h. The circadian bioluminescent phenotype has also proven useful in beginning to dissect the pathways from photoreceptors to the circadian oscillator that are ultimately used to entrain the clock (Millar et al., 1995b). By crossing the *CAB:luc* marker into a variety of photoreceptor mutants (e.g. *hy1*), as well as photomorphogenetic mutants (*det1* and *cop1*), the effects of these mutations on circadian period could be easily assayed. It was demonstrated that distinct pathways emanating from activation of phytochromes and a blue light photoreceptor converge on downstream genes such as *det1* before inputting into the clock to shorten period under continuous illumination. This is the first genetic dissection of circadian input in any organism, and the use of more specific mutants in the future will precisely define the photoreceptor interactions used to entrain the clock under natural conditions.

In addition to the genetic studies outlined above, advances have been made in understanding circadian-regulated gene expression, as a model for clock output. It has been demonstrated that phytochrome and the circadian clock utilize distinct DNA-protein interactions to control CAB transcription (Anderson et al., 1995). Furthermore, a circadian-regulatory element (CCRE) has been located on a 35bp element within the proximal CAB promoter (Carre and Kay, 1995), the first time such an element has been defined in any organism. It is clear that *Arabidopsis* as a model system has much to contribute to the understanding of these ubiquitous biological clocks (Kay and Millar, 1995). Anderson SL, Kay SA (1995) Function dissection of circadian clock- and phytochrome-regulation of CAB gene transcription. Proc. Natl. Acad. Sci. USA 92: 1500-1504.

Carre I, Kay SA (1995) Multiple DNA-protein interactions at a circadian-regulated promoter element. Plant Cell 7: 2039-2051.

Kay SA, Millar AJ (1995) New models in vogue for circadian clocks. Cell 83: 361-364.

Millar AJ, Carre I, Strayer C, Straume M, Chua NH, Kay SA (1995a) Identification of circadian mutants in *Arabidopsis* by luciferase imaging. Science 267: 1161-1163.

Millar AJ, Chory JC, Chua NH, Kay SA (1995b) Regulation of circadian period by phototransduction pathways in *Arabidopsis*. Science 267: 1163-1166.

Photoregulation

Much of our current information on how plants perceive and respond to their light environment is derived from studies with *Arabidopsis*. These studies have identified the basic genetic framework of light perception and transduction that is likely to be common to all higher plants. The genetic analysis indicates that light responses are not simply endpoints of linear signal transduction pathways, but are the result of the integration of information from a variety of receptors acting through a complex network of interacting signaling components. Approximately 40 *Arabidopsis* genes (published and unpublished results) have been identified that play a role in this signal transduction network. These genes define the photoreceptors themselves, as well as positive and negative regulatory elements that act downstream from these photoreceptors.

The genetic and molecular studies suggest a simple model for light-regulated development in which the action of multiple photoreceptors is integrated through global repressors (*DET, COP, FUS*) which then act through specific regulators (e.g., *DET3, DOC1*) to repress gene expression and morphogenesis in dark-grown seedlings. When this repression is relieved, cell-type specific positive regulators (e.g., *CUE1*) can act to induce gene expression and development. Though this model is simplistic and does not address the actual mechanisms involved, it suggests a framework with which to address the mode of action and the interactions

of the various gene products.

In addition to the genetic studies, a significant result was obtained this year in the area of blue light perception (Lin et al., 1995a,b). *HY4* was shown unequivocally to encode a blue light photoreceptor. Studies from Anthony Cashmore's lab (U. of Pennsylvania) showed that the *HY4* gene encodes a 75 kD flavoprotein that noncovalently binds stoichiometric amounts of flavin adenine dinucleotide. In addition, this lab showed that overexpression of the *HY4* gene product in transgenic tobacco resulted in hypersensitivity to blue, UV-A, and green light, which explains the broad action spectrum for responses mediated by this photoreceptor in *Arabidopsis*. They have proposed that the *HY4* gene product be renamed CRY1, after cryptochrome, the name commonly given to plant blue/UV-A photoreceptors.

An interesting result suggesting a mechanism for the transduction of light signals was published this year (von Arnim and Deng, 1994). In this study, the authors present evidence that the negative regulator, COP1, becomes nuclear localized in dark-grown hypocotyl cells or in roots from light-grown plants. In contrast, COP1 is more cytoplasmically localized in light-grown hypocotyl cells. The authors propose that COP1 exerts its repressive activity in the dark by regulated translocation to the nucleus, whereas in the light, COP1 becomes depleted from the nuclear compartment.

Lin C, Ahmad M, Gordon D, Cashmore AR (1995a) Expression of an *Arabidopsis* cryptochrome gene in transgenic tobacco results in hypersensitivity to blue, UV-A, and green light. Proc. Natl. Acad. Sci. USA 92: 8423-8427.

Lin C, Robertson D, Ahmad M, Raibekas A, Jorns M, Dutton P, Cashmore AR (1995b) Association of flavin adenine dinucleotide with the *Arabidopsis* blue light receptor CRY1. Science. 269: 968-970.

von Arnim A, Deng X-W (1994) Light inactivation of *Arabidopsis* photomorphogenic repressor COP1 involves a cell-specific regulation of its nucleocytoplasmic partitioning. Cell 79: 1035-1045.

Cell Biology

The organelles of the plant secretory system and the plasma membrane each contain a specific complement of resident integral membrane proteins, which provide each organelle with some of their unique characteristics. Over the past year, genes encoding some of these proteins have been isolated and characterized and this has allowed the function and localization of the encoded proteins to be determined.

Soluble resident proteins of the endoplasmic reticulum contain a Cterminal tetrapeptide motif which mediates their localization in this organelle. As some of these proteins are modified by enzymes found in the Golgi apparatus, it has been suggested that they can escape from the ER and are retrieved by a specific sorting mechanism. A receptor protein (Erd2p) was identified in yeast which is found at the cisGolgi and may bind to and retrieve ERresident proteins back to the ER. An *Arabidopsis* homologue of the *ERD2* gene has been cloned (called *aERD2*) and encodes a highly hydrophobic protein with seven putative transmembrane domains (Bar-Peled et al., 1995). The *aERD2* gene is able to complement a yeast *erd2* mutant, indicating that the yeast and *Arabidopsis* proteins have similar functions and that a*Erd2p* may also be located at the cisGolgi. Varying levels of expression of the *ERD2* gene are observed in different tissue and cell types. The highest levels of *ERD2* mRNA are found in roots. Very low transcript levels are observed in leaves throughout development, but with an increased accumulation of transcript in trichomes in older leaves. This could reflect differing levels of secretory pathway activity in roots and leaves. Stress conditions affecting the secretory pathway (tunicamycin treatment and cold shock) cause an increase in expression of *aERD2*. This suggests that the secretory pathway can be regulated in response to environmental conditions.

Syntaxins are integral membrane proteins, thought to act as receptors for transport vesicles arriving at and fusing with the membrane, with the bulk of the protein facing the cytosol. Upon docking of a vesicle with its target membrane, syntaxin interacts with a number of proteins, both in the vesicle membrane and in the cytosol to form a docking complex. Different isoforms of syntaxin have been proposed to reside on different cellular membranes and to provide specificity for the docking and fusion reaction. A yeast *pep12* mutant is defective in the targeting of proteins to the vacuole and Pep12p is thus likely to function in vesicle transport between the trans Golgi network and the vacuole. An *Arabidopsis* cDNA (*aPEP12*) was isolated by functional complementation of the yeast *pep12* mutant and found to be homologous to the yeast *PEP12* gene and other members of the syntaxin family (Bassham et al., 1995). *aPep12p* is, therefore, a potential component of the plant vacuolar transport machinery.

In plants, the primary active transport system at the plasma membrane is the H+-ATPase, which couples ATP hydrolysis to the transport of protons from the cytosol across the plasma membrane. This produces a membrane potential consisting of an electrical potential (negative inside) and a pH gradient (more acidic outside) which can be used to drive the uptake of solutes into the cell. cDNA and genomic clones encoding H+-ATPases have been isolated from a number of species, with the most extensive analysis performed in *Arabidopsis.* Surprisingly, a large family of genes (AHAs, for *Arabidopsis* H+-ATPase) encoding H+-ATPases have been identified, and differential regulation of mRNA expression for these genes has been demonstrated in *Arabidopsis* and tobacco (Michelet et al., 1994). H+-ATPase clones from both of these plants have been found to contain a short open reading frame in the 5' region upstream of the main reading frame, which may be involved in translational gene regulation. Developmental and growth conditions have also been seen to regulate H+-ATPase expression, as has cytosolic calcium concentration (Kinoshita et al., 1995). The expression of some plant genes in yeast demonstrated that they do encode functional H+-ATPases with properties similar to the yeast plasma membrane H+-ATPase. A comparison between the properties of AHA1, 2 and 3 expressed in yeast has shown that these three isoforms display different pH optima, Km's for ATP, and inhibitor sensitivities, suggesting that there are functional differences between the isoforms.

It has been hypothesized that each AHA isoform is expressed in a particular transport tissue and thus could create the driving force for solute transport in that tissue. This has been demonstrated recently for the AHA3 protein, which was found by immunocytochemical localization to be restricted to the plasma membrane of phloem companion cells and, therefore, may be involved in phloem loading (de Witt and Sussman, 1995).

The plant vacuolar membrane also contains another electrogenic proton pump, the H+-translocating pyrophosphatase (H+-PPase). Inorganic pyrophosphate is used as the energy source for the translocation of protons across the membrane, and H+- translocation is dependent on the presence of K+ in the cytosol. In contrast to the H+-ATPase, the H+-PPase appears to consist of a single approximately 80 kDa subunit; the expression in yeast of this subunit from *Arabidopsis* demonstrated that it is sufficient for all known catalytic functions of the enzyme (Kim et al., 1994).

A family of proteins that has received much attention recently in plants is the aquaporins, which mediate the passage of water across membranes. They are part of a family of related proteins, the MIP family, which all appear to consist of six membranespanning domains with their amino and carboxytermini facing the cytosol. Whereas the aquaporins transport water, other members of this family transport solutes such as ions and glycerol. In plants, a number of *MIP* homologues have been identified in both the plasma membrane and tonoplast. The function of a *MIP* homologue in plants was addressed by expressing an antisense construct of the blue light inducible *AthH2* gene of *Arabidopsis* (Kaldenhoff et al., 1995). Leaf protoplasts from the antisense plants show reduced water uptake when compared with control plants.

BarPeled M, da Silva Conceicao A, Frigerio L, Raikhel NV (1995) Expression and regulation of *aERD2*, a gene encoding the KDEL receptor homolog in plants, and other genes encoding proteins involved in ERGolgi vesicular trafficking. Plant Cell 7: 667676

Bassham DC, Gal S, da Silva Conceicao AD, Raikhel NV (1995) An *Arabidopsis* syntaxin homologue

isolated by functional complementation of a yeast *pep12* mutant. Proc. Natl. Acad. Sci. USA 92: 72627266.

DeWitt ND, Sussman MR (1995) Immunocytological localization of an epitopetagged plasma membrane proton pump (H+-ATPase) in phloem companion cells. Plant Cell 7: 2053-2067.

Kaldenhoff R, Kolling A, Meyers J, Karmann U, Ruppel G, Richter G (1995) The blue lightresponsive *AthH2* gene of *Arabidopsis thaliana* is primarily expressed in expanding as well as in differentiating cells and encodes a putative channel protein of the plasmalemma. Plant J. 7: 87 95.

Kim EJ, Zhen RG, Rea PA (1994) Heterologous expression of plant vacuolar pyrophosphatase in yeast demonstrates sufficiency of the substratebinding subunit for proton transport. Proc. Natl Acad. Sci. USA 91: 6128 6132.

KinoshitaT, Nishimura M, Shimazaki K.i (1995) Cytosolic concentration of Ca2+ regulates the plasma membrane H+-ATPase in guard cells of fava bean. Plant Cell 7: 13331342.

Michelet B, Lukaszewicz M, Dupriez V, Boutry M (1994) A plant plasma membrane protonATPase gene is regulated by development and environment and shows signs of a translational regulation. Plant Cell 6: 13751389.

Biochemistry

Biochemical mutants of *Arabidopsis* are revealing important information about a wide variety of questions that are of fundamental importance to plant biologists, and also have implications for industrial uses of plants. Because removal of lignin is expensive and environmentally destructive, it would be desirable to be able to manipulate the amount and type of lignin made by pulp trees. Clint Chapple and colleagues at Purdue University have cloned a gene for an enzyme of lignin biosynthesis. The *FAH1* gene encodes ferulate-5-hydroxylase (F5H), which is a cytochrome P450-dependent monooxygenase of the general phenylpropanoid pathway that is essential for the production of syringyl lignin in all angiosperms. A prerequisite to this goal is to understand how the lignin biosynthetic pathway is normally regulated. To this end, Chapple and colleagues are testing the hypothesis that F5H is a major control point in the phenylpropanoid pathway. They have engineered transgenic *Arabidopsis* plants that overexpress the F5H gene under the CaMV 35S promoter. Analysis of these plants will reveal whether manipulating F5H expression has an impact on lignin composition. In the long run, such experiments may lead to the production of transgenic trees that can be more efficiently utilized by pulp mills for the production of paper products.

In addition to the production of lignin, the general phenylpropanoid pathway is responsible for synthesis of a large and diverse collection of aromatic secondary metabolites, including hydroxycinnamic acids and flavonoids. While these compounds are proposed to be important in many important aspects of plant physiology, in many cases definitive information is lacking. The *fah1* mutant (Chapple et al., 1992) not only has no syringyl lignin, but it also is missing sinapoyl malate, a major soluble UV-absorptive sunscreen of *Arabidopsis*. As a result, *fah1* is highly sensitive to the damaging effects of UV-B (Landry et al., 1995) , and much more so than the flavonoid-deficient *tt4* chalcone synthase mutant. This result indicates that, although flavonoids are generally regarded as the key sunscreens of plants, the role of hydroxycinnamate compounds as UV-B protectants should not be discounted.

Flavonoids have a well documented role in pollen function in plants as diverse as petunia and maize. Therefore, the observation that *Arabidopsis* flavonoid deficient mutants do not have any apparent fertility defect has been regarded as enigmatic. A plausible hypothesis was that the flavonoid deficient mutants are leaky, and enough flavonoids accumulate in pollen to allow normal fertility. Brenda Shirley and colleagues at

Virginia Tech have critically challenged this idea by studying the *tt4-2YY6* mutant, which has a defect in the only known gene for *Arabidopsis* chalcone synthase, the committing enzyme of flavonoid biosynthesis. They accumulated considerable evidence that this mutant does not make chalcone synthase, including findings that all of the chalcone synthase transcripts in seedlings and flowers are aberrantly spliced. Considering that this mutant is male fertile, it appears that some plants do not require flavonoids for normal pollen function.

There is considerable interest in using plants for bioremediation of polluted areas, especially heavy metal contaminated sites. Recent work in the laboratory of Christopher Cobbett (University of Melbourne) on heavy metal detoxification in *Arabidopsis* may provide leads on how to optimize this process. Cobbett and coworkers have obtained mutants that are unable to grow in the presence of cadmium (II), and have shown that these are defective in the synthesis of phytochelatins, which are synthesized in response to heavy metals and form complexes with such metals (Howden et al., 1995a; Howden et al., 1995b) . The *cad1* mutant is defective in phytochelatin synthase, the final enzyme in phytochelatin biosynthesis. In contrast, *cad2* is deficient in synthesis of the phytochelatin precursor glutathione. The availability of phytochelatin-deficient mutants should provide opportunities for discovering other mechanisms of heavy metal detoxification, and leads for making phytoremediation more efficient. Studies of the *cad2* mutant should also provide needed information about oxidative stress responses in plants, because glutathione is considered an important regulator of the oxidation state of the plant cell, in addition to its role in heavy metal detoxification.

One of the most economically important single gene modifications known is a spontaneous mutation in rapeseed that prevents synthesis of the toxic fatty acid erucic acid. This mutation converts a non-edible oil into an edible oil of the highest quality and is the basis for the development of Canola as a crop species. The elongase complex responsible for synthesis of erucic acid has been intractable to conventional biochemical approaches. During the past year, the first component of the elongase was cloned by transposon tagging in *Arabidopsis* and shown to have sequence similarity to several condensing enzymes and beta keto acyl ACP synthase (James et al., 1995). Parallel work by Jim Metz and colleagues at Calgene showed that a homolog of this gene functionally complements the mutation in Canola. Also comparison of the *FAE1* sequence to the database of more than 21,000 partially sequenced cDNA clones from *Arabidopsis* revealed that *Arabidopsis* contains a small multigene family of related enzymes. Presumably, these genes encode enzymes involved in the synthesis of long chain acyl groups that comprise the surface wax of epidermal cells.

Chapple CS, Vogt T, Ellis BE, Somerville CS (1992) An *Arabidopsis* mutant defective in the general phenylpropanoid pathway. Plant Cell 4: 1413-1424.

Howden R, Andersen CR, Goldsbrough PB, Cobbett CS (1995a) A cadmium-sensitive, glutathione-deficient mutant of *Arabidopsis thaliana*. Plant Physiol. 107: 1067-1073.

Howden R, Goldsbrough PB, Andersen CR, Cobbett CS (1995b) Cadmium-sensitive, *cad1* mutants of *Arabidopsis thaliana* are phytochelatin deficient. Plant Physiol. 107: 1059-1066.

James DW, Lim E, Keller J, Plooy I, Ralston E, Dooner H (1995) Directed tagging of the *Arabidopsis FATTY ACID ELONGATION 1 (FAE1)* gene with the maize transposon activator. Plant Cell 7: 309-319.

Landry LG, Chapple CCS, Last RL (1995) *Arabidopsis* mutants lacking phenolic sunscreens exhibit enhanced ultraviolet-B injury and oxidative damage. Plant Physiol. 109: 1159-1166.

Response to Pathogens

Arabidopsis researchers have continued to build upon the seminal breakthroughs of 1994 (reviewed in Dangl, 1995; Staskawicz et al., 1995) and made important strides forward in understanding the molecular basis of

plant disease resistance. The most critical issues to address in this field are: How do plants recognize pathogens? And, how is that recognition translated into a successful resistance response? Answers to these questions will be of benefit to both fundamental and applied plant biology.

Important new data published in 1995 impinge on both questions. Cloning of the *RPM1* resistance (*R*) gene revealed that although it shares predicted protein similarity with previously published resistance genes such as the *Arabidopsis RPS2* gene, it encodes resistance to bacterial pathogens expressing either of two different molecules which trigger disease resistance (Grant et al., 1995). This contrasts with the classical model of a single pathogen trigger molecule interacting with a single *R* gene product. Generally, the nature of the pathogen ligand and how it initiates the disease resistance pathway remain unsolved, as does identification of that portion of the *R* gene product responsible for specificity.

Signal transduction events subsequent to pathogen recognition are also being investigated using genetic screens in *Arabidopsis*. These screens are varied and measure loss of specific *R* gene function (Century et al., 1995); inability to induce systemic acquired resistance (Delaney et al., 1995); and lack of, or constitutive, activation of a defense gene promoter-reporter transgene (Bowling et al., 1994; Cao et al., 1994). Building on last year's demonstration that salicylic acid (SA) accumulation is a key component of disease resistance in *Arabidopsis* (Delaney et al., 1994), the new mutants can often be distinguished on the basis of whether they function in a specific *R* gene-dependent pathway, or are generally required for disease resistance. Several classes of mutants have now been identified. They define loci required for the action of a number of *R* genes, but which apparently act upstream of the requirement for salicylic acid accumulation (Century et al., 1995), and those that act downstream of SA accumulation (Cao et al., 1994; Delaney et al., 1995). Importantly, these last two mutants define genes required for all resistance reactions and the ability of the plant to establish systemic acquired resistance. These loci thus encode key regulators of the resistance pathway.

Finally, in an enticing example of how *Arabidopsis* research can add greatly to general knowledge of how pathogens interact with their hosts, it was shown this year that a bacterial pathogen of mammals is also an *Arabidopsis* pathogen and that some of the pathogenicity factors required for infection of mammals are also required to infect plants (Rahme et al., 1995). This result suggests the exciting possibility that evolutionary conservation of pathogenicity factors will be mirrored by conservation of the recognition and signal transduction events required to protect the host.

Bowling SA, Guo A, Cao H, Gordon AS, Klessig DF, Dong X (1994) A mutation in *Arabidopsis* that leads to constitutive expression of systemic acquired resistance. Plant Cell 6: 1845-1857.

Cao H, Bowling SA, Gordon S, Dong X (1994) Characterization of an *Arabidopsis* mutant that is non-responsive to inducers of systemic acquired resistance. Plant Cell 6: 1583-1592.

Century KS, Holub EB, Staskawicz BJ (1995) *NDR1*, a locus of *Arabidopsis thaliana* that is required for disease resistance to both a bacterial and a fungal pathogen. Proc. Natl. Acad. Sci. USA 92: 6597-6601.

Dangl JL (1995) Piécè de Resistance: Novel classes of plant disease resistance genes. Cell 80: 363-366.

Delaney T, Uknes S, Vernooij B, Friedrich L, Weymann K, Negrotto D, Gaffney T, Gut-Rella M, Kessman H, Ward E, Ryals J (1994) A central role of salicylic acid in plant disease resistance. Science 266: 1247-1250.

Delaney TP, Friedrich L, Ryals JA (1995) *Arabidopsis* signal transduction mutant defective in chemically and biologically induced disease resistance. Proc. Natl. Acad. Sci. USA 92: 6602-6606.

Grant MR, Godiard L, Straube E, Ashfield T, Lewald J, Sattler A, Innes RW, Dangl JL (1995) Structure of the *Arabidopsis RPM1* gene enabling dual specificity disease resistance. Science 269: 843-846.

Rahme LG, Stevens EJ, Woolfort SF, Shao J, Tompkins RG, Ausubel FM (1995) Common virulence factors

for bacterial pathogenicity in plants and animals. Science 268: 1899-1902.

Staskawicz BJ, Ausubel FM, Baker BJ, Ellis J, Jones JDG (1995) Molecular genetics of plant disease resistance. Science 268: 661-667.

Response to Environmental Stress

This past year has seen significant progress in our understanding of the regulation of gene expression in *Arabidopsis* exposed to various abiotic stresses such as ozone, UV-irradiation, touch, cold and anoxia. A variety of results reported at the *Arabidopsis* Meeting in Madison augment a theme that has been developing over the past several years; namely, that different stress responses appear to be regulated by overlapping and interacting signal transduction pathways. For example, work in Keith Davis' lab (Ohio State University) has demonstrated that the pattern of gene expression observed in ozone-treated plants overlapped significantly with the pattern of gene expression observed during a hypersensitive response to pathogen attack. For example, ozone induces an active defense response, correlated with salicylic acid accumulation, that is capable of restricting bacterial growth in infiltrated leaves. There has also been significant progress in studying the genetics of DNA repair in *Arabidopsis*. Recent results from Anne Britt's laboratory (University of California, Davis) indicate that there is an active, light dependent repair pathway for UV-induced photoproducts and that it is possible to isolate mutants deficient for this repair pathway. Preliminary studies indicate that a second pathway for the repair of cyclobutyl dimers is present and may be most active on actively transcribed DNA sequences. In other work presented at the *Arabidopsis* meeting, Kathleen Smith and Ethan Signer (Massachusetts Institute of Technology) discussed the possible function of a novel *Arabidopsis* gene, *RAD51*. *RAD51* is a homolog of the yeast *ScRAD51* gene and the bacterial *RecA* gene, and thus may be involved in the repair of double strand breaks in the genome. *RAD51* transcripts were found at very low levels in most tissues. *RAD51* mRNA was rapidly and strongly induced by exposure of gamma-irradiation in a dose-dependent manner. Interestingly, 2 mutants isolated by Corrine Davies (University of Arizona) which are hypersensitive to gamma-irradiation do not exhibit the induction of *RAD51* in response to irradiation, supporting the suggestion that *Arabidopsis RAD51* may be involved in DNA repair. Recent work presented by Mike Thomashow (Michigan State University) has shown that *Arabidopsis* is a useful model for studying cold tolerance. Thomashow and his co-workers have found that the cold-regulated *COR15a* gene of *Arabidopsis* encodes a polypeptide that is targeted to the stromal compartment of chloroplasts and that constitutive expression of the *COR15a* gene results in the enhancement of chloroplast freezing tolerance in non-acclimated plants.

Janet Braam (Rice University) summarized her group's efforts to characterize the regulation and function of the 'touch' (*TCH*) genes. Highlights included the determination that a subset of the touch genes are induced by cold shock and that changes in cytoplasmic calcium levels may be involved in this induction. Further studies of the *TCH3* and *TCH4* genes revealed that TCH3, a putative calcium-binding protein, accumulates during development at sites that may be under mechanical stress or that are undergoing expansion. *TCH4* appears to encode a xyloglucan endotransglycosylase (XET), and as such, may have an important role in modifying the cell wall during responses to environmental stimuli and during development.

John Sedbrook (University of Wisconsin) summarized work from Patrick Masson's laboratory on the plant response to anoxia. This group has utilized transgenic *Arabidopsis* plants expressing Aequorin, a sensitive monitor for changes in free calcium levels, to examine the potential role of calcium in regulating the changes in gene expression associated with anoxia. AEQUORIN-expressing *Arabidopsis* seedlings developed a biphasic luminescence response in cotyledons and leaves, but not in roots or hypocotyls. This luminescence response is composed of a fast and transient first peak which occurs within minutes of anoxia, followed by a second prolonged luminescence response which lasts 1.5 to 4 hours. Studies with calcium inhibitors indicate that these changes involve modulation of both intracellular and extracellular calcium stores. The inhibitor

sensitivity of calcium changes associated with the return to normoxia are consistent with the formation of oxygen free radicals, thus adding anoxia as yet another stress that may utilize active oxygen species as signal molecules.

Examples of recent papers in this area of research are listed below:Antosiewicz DM, Polisensky DH, Braam J (1995) Cellular localization of the Ca2+ binding TCH3 protein of Arabidopsis. Plant J. 8: 623-636.Chen J-J, Mitchell D, Britt AB (1994) A light-dependent pathway for the elimination of UV-induced pyrimidine [6-4] pyrimidinone photoproducts in *Arabidopsis thaliana*. Plant Cell 6: 1311-1317.Polisensky DH, Braam J (1996) Cold shock regulation of the Arabidopsis TCH genes and the effects of modulating intracellular calcium levels. Plant Physiol: (in press).Sharma YK, Davis KR (1994) Ozone-induced expression of stress-related genes in *Arabidopsis thaliana*. Plant Physiol. 105: 1089-1096.Sharma YK, Davis KR (1995) Isolation of a novel ozone-induced cDNA in *Arabidopsis* by differential display. Plant Mol. Biol. 29: 91-98.Sharma Y, Raskin I, Davis KR (1996) Ozone-induced responses in *Arabidopsis thaliana*: the role of salicylic acid in the accumulation of defense-related transcripts and induced resistance. Proc.Natl. Acad. Sci., USA: (in press) Sistrunk ML, Antosiewics DM, Purugganan MM, Braam J (1994) *Arabidopsis TCH3* encodes a novel Ca2+ binding protein and shows environmentally induced and tissue-specific regulation. Plant Cell 6: 1553-1565.Sonti RV, Chiurazzi M, Wong D, Davies CS, Harlow GR, Mount DM, Signer ER (1995). *Arabidopsis* mutants deficient in T-DNA integration. Proc. Natl. Acad. Sci. USA 92: 11786-11790.Xu W, Purugganan MM, Polisensky DH, Antosiewicz DM, Fry SC, Braam J (1995) *Arabidopsis TCH4*, Regulated by hormones and the environment, encodes a xyloglucan endotransglycosylase. Plant Cell 7: 1555-1567.

NATIONAL AND TRANSNATIONAL PROJECTS:

Arabidopsis research continues to blossom in many different parts of the world. The international nature of this effort is apparent from recent stock center transactions, which included requests for *Arabidopsis* materials from Argentina, Australia, Belgium, Brazil, Canada, Chile, China, Colombia, Denmark, Finland, France, Germany, Hong Kong, Hungary, Iceland, Israel, Italy, Japan, Korea, Luxembourg, Malaysia, Mexico, the Netherlands, New Zealand, Norway, Poland, Portugal, Russia, Singapore, South Africa, Spain, Sweden, Taiwan, Turkey, Ukraine, the United Kingdom, and the United States. The following summaries provide examples of recent advances in regional centers of *Arabidopsis* research. Additional information on these programs can be obtained from the appropriate contact person.

Australia

Several research groups in Canberra and Melbourne focus on *Arabidopsis* as a model system, with recent additions to this community extending across the Tasman Sea to Auckland, New Zealand. Local support for the *Arabidopsis* Genome Project has been provided by the CSIRO Division of Plant Industry and The Research School of Biological Sciences at the Australian National University. Both of these institutes are located in Canberra. The research projects under study largely involve mutant hunts designed to identify genes of interest, with gene isolation, sequencing, and characterization the ultimate aim. The genes in question are associated with a wide range of functions, including the regulation of male and female fertility, flowering time, floral morphogenesis, response to heavy metals, and the involvement of cellulose in generating cell shape in roots. Several YAC libraries are now held at CSIRO, and clones are screened and distributed to local *Arabidopsis* workers upon request. Recent highlights of *Arabidopsis* research in Australia include a publication in PNAS on the effect on fertility of perturbing the expression of a gene normally active in both the tapetum and microspores, and two papers in Plant Physiology, along with a cover

photograph, on cadmium sensitivity in *Arabidopsis*. In September, the Annual General Meeting of the Australian Society of Plant Physiologists in Sydney welcomed several international *Arabidopsis* workers as Keynote speakers. The research community in Australia is a relatively small, geographically isolated group, but electronic mail and the free exchange of seeds, clones, and sequence data have greatly facilitated the establishment of active programs.

Contact Person: David Smyth, Monash University

Email: david.smyth@sci.monash.edu.au

Belgium

This was an important year for biotechnology in Belgium, and specifically in Flanders, as evidenced by the government establishment of a "Flanders Interuniversity Institute for Biotechnology". The core activities of this institute are defined by four research groups, including the Laboratory of Plant Genetics in Ghent, a recognized center for plant research and studies with *Arabidopsis*. An important aspect of this institute is fostering positive interactions with private industry. Apart from the foundation of this Institute, the Belgian and Flemish governments continued to support several projects, including those dealing with hormone action and stress, that further the development of *Arabidopsis* as a model plant. Progress was also made towards the establishment of a physical map of *Arabidopsis* chromosome III. In addition, the Belgian government continued to support a number of research collaborations among the universities. For example, the Laboratory of Genetics in Ghent interacts with universities in Antwerp and Brussels in a national program on plant genetics. A very promising aspect of this program has been the establishment of a protein database of *Arabidopsis* using 2-dimensional electrophoresis and partial amino acid sequence analysis.

Contact Person: Marc Van Montagu, University of Ghent

Email Address: aruyt@gengenp.rug.ac.be

Canada

Although no funding program in Canada specifically supports research with *Arabidopsis*, significant progress continues to be reported in selected laboratories located throughout the country using *Arabidopsis* as a model system. Projects focus on a wide range of topics such as seed maturation, triacylglyceride biosynthesis, phosphate transport, herbicide resistance, flower development, plant pathogenesis, meiosis, and vegetative development. *Arabidopsis* was also a focus of discussion at a Canadian workshop on plant molecular biology held in Calgary (March, 1995) and a French/Canadian Jacques Cartier Conference on metabolism and plant development held in Lyon (December, 1995). Several laboratories and companies with interests in *Brassica* species have also benefited from research with *Arabidopsis*. For example, Maurice Moloney (University of Calgary) has demonstrated that oleosins can be used as carriers for the production of foreign peptides in seeds, and based on initial studies with *Arabidopsis* as a model, has formed a company (SemBioSys) that currently employs 12 full-time research staff. Bertrand Lemieux (York University) is continuing his collaboration with Ronald Davis (Stanford University) to map EST sites by PCR amplification. Support for this project is being provided by a 2-year Canadian Genome Analysis and Technology grant. To date, Deval Lashkari at Stanford has synthesized over 3,000 oligonucleotides for this effort, 90% of which amplify single copy sequence genes, and 260 ESTs have been anchored to at least one YAC clone. Over 1,000 ESTs should

be assigned to YAC clones by September, 1996.

Contact Person: Bertrand Lemieux, York University

Email Address: fs300500@sol.yorku.ca

European Union

This is an important time for plant science research in Europe. The Framework 4 budget for plant biology is the largest ever and there are significant opportunities to establish strong networks of labs for large-scale biological work. For example, sequencing projects can provide information directly to networks designed to search for specific functions, which in turn can be linked to projects that aim to understand fundamental aspects of plant development and metabolism that are important for crop improvement. This superstructure is currently being assembled with *Arabidopsis* as the principal organism, based on the example of networks established previously for yeast. Genome sequencing teams are already in place and have sufficient experience and material available to tackle large regions of the genome. Their only limitation at present is resources. Three function search networks have been set up and will start work at the end of 1995. One of these, the *Arabidopsis* Insertional Mutagenesis System (AIMS project) will produce sufficient T-DNA and transposon insertions to make multiple insertions in every gene. Another team is specifically targeting the FCA region on chromosome 4 for insertions using Ac/Ds and En transposons. Another project, in collaboration with AIMS, will isolate T-DNA and transposon insertions in members of the large Myb transcription factor gene family, of which over 80 members have been partially sequenced. If successful, other genes and large gene families will be targeted. The third network will characterize membrane proteins at the sequence and functional level. Allied to this is further development of the Nottingham Stock Centre and large-scale comparative genome mapping between *Arabidopsis*, *Brassica* species, and rice. In addition, selected projects have been outlined to isolate genes of agronomic importance from *Arabidopsis*, with the emphasis on source-sink relations, photomorphogenesis, plant architecture, flowering time, root development, and cell wall architecture.

Contact Person: Michael Bevan, John Innes Centre

Email Address: bevan@bbsrc.ac.uk

France

At least 15 laboratories in France are continuing to use *Arabidopsis* for studies of embryogenesis, abscisic acid signaling, seed dormancy, ion transport, root development, lipid metabolism, cell cycle control, protein kinases, gametogenesis, plasmalemma proteins, nitrate and carbon metabolism, transposable elements, plant-pathogen interactions, stomatal regulation, and genome structure. In addition, large-scale collaborative projects involving genome analysis are continuing in the areas of EST/cDNA sequencing, genomic sequencing, physical mapping and YAC library characterization, and T-DNA insertional mutagenesis. Nine French laboratories have analyzed over 7,000 EST sequences representing 5,000 clones obtained from 10 different libraries. Sequences from both 5' and 3' ends are now available for more than 2,000 clones. Over 5,200 non-redundant sequences have been submitted to GenBank and dbEST. Clones are regularly sent to ABRC at Ohio State University. The French genome agency (GREG) currently supports full-length sequencing of selected cDNAs and classification of ESTs based on patterns of expression. In the area of

genomic sequencing, four laboratories (Delseny, Lescure, Mache, Kreis) are currently participating in the ESSA program on a 25 Kb/year basis. Mapping of ESTs and ordering of the CIC YAC library is also continuing with GREG support. A database of this library has been established and is managed by D. Bouchez (INRA-Versailles). Characterization of T-DNA lines is also continuing in the laboratory of G. Pelletier (INRA-Versailles). T3 stocks of 12,000 lines are currently being prepared for distribution through the Nottingham Stock Centre. Techniques are also being developed to screen the collection for insertions in genes of known sequences. Advances in cDNA sequencing and YAC library characterization are summarized in two recent papers (Cooke et al.; Creusot et al.; Plant J. 1995).

Contact Person: Michel Caboche, INRA, Versailles

Email Address: caboche@versailles.inra.fr

Germany

Arabidopsis research is now well-established in Germany, both at universities and national institutes for plant research. A special research program (*Arabidopsis* as a Model for the Genetic Analysis of Plant Development) funded by the German Research Foundation (DFG) has been established to link research efforts at different locations. This program recently entered its second funding period. Thirty groups applying for funding within this program presented their work at a meeting held in May, 1995 at Bad Honnef (near Bonn). Topics of discussion included gametophyte development, embryogenesis, seed maturation, shoot meristem formation, leaf and trichome initiation, and developmental responses controlled by light and hormones. Participants at this meeting elected Rick Walden (MPI, Cologne) to replace Jeff Dangl (now at the University of North Carolina) as co-coordinator of the research program. The DNA resource center that Jeff established with European funding will now be fully funded by DFG and maintained by Csaba Koncz (MPI, Cologne). In addition, an information network has been established by Toni Schaeffner (University of Munich) to facilitate exchange between groups. Future meetings are planned for the group to discuss the yeast two-hybrid system in relation to studies of protein interactions, and saturation "knock-out" mutagenesis in *Arabidopsis*. Other research groups funded outside the program work on a variety of physiological questions, including heat shock responses, transport and partitioning of carbon, water transport, and protein secretion.

Contact Person: Gerd Jurgens, University of Tubingen

Email Address: geju@fserv1.mpib-tuebingen.mpg.de

Italy

Approximately nine laboratories located at Universities, Institutes of the Consiglio Nazionale delle Ricerche, and the National Institute of Nutrition, are currently involved in *Arabidopsis* research. Subjects under study include mutational and molecular analyses of root development and nutations, photoinhibition, fusicoccin resistance, gravitropism, signal transduction pathways, transcription factors, and ABA-regulated gene expression. Most of the laboratories involved in this work are located in Milan, Rome, Monterotondo, and Parma. One laboratory in Naples is scheduled to participate in the European Program to produce large-scale genomic sequence from chromosome 4. The primary funding sources for *Arabidopsis* research are the Consiglio Nazionale delle Ricerche, the Ministry of Agricultural Resources, and the European Commission.

A meeting of all people interested in *Arabidopsis* research is planned for early in 1996. At this time, the organization of an Italian *Arabidopsis* Network will be attempted.

Contact Person: Fernando Migliaccio, IBEV Institute / Consiglio Nazionale delle Ricerche, Monterotondo (Rome)

Email Address: miglia@nserv.icmat.mlib.cnr.it

Japan

Nearly 40 laboratories in Japan use *Arabidopsis* as a major experimental plant for genetic studies on flower and leaf development, responses to physical and chemical stimuli, root morphology, virus infection, hormonal and transcriptional regulation, shoot and root regeneration from callus tissues, and the isolation and characterization of genes involved in heat shock responses, desiccation, lipid biosynthesis, signaling pathways, protein phosphorylation, and the regulation of transcription. During the past year, many laboratories examined the expression of isolated genes of interest in transgenic plants. Other efforts involved the construction of transgenic plants for the purpose of identifying tagged mutants. Recent scientific activities of *Arabidopsis* researchers in Japan were reported at several workshops and symposia, including annual meetings of The Botanical Society of Japan, The Genetics Society of Japan, The Japanese Society of Molecular Biology, and The Japanese Society of Plant Physiologists. More than 90 participants attended the 5th workshop on *Arabidopsis* studies (organized by K. Okada and Y. Shimura) held at the National Institute for Basic Biology at Okazaki in November, 1994. Nearly 150 participants attended the 6th workshop on *Arabidopsis* studies (organized by A. Oka, T. Aoyama, and K. Goto) held at the Institute for Chemical Research at Kyoto University in November, 1995. Talks presented at these workshops covered mutant analyses, transformation, transcriptional regulation, and gene cloning. A communication network for the *Arabidopsis* community in Japan was established in January, 1995. This network of more than 150 members is known as nazuna-net, after the Japanese word for wild Brassica, and facilitates the exchange of information on research activities as well as upcoming meetings and workshops. Details about this network can be obtained from Takayuki Kohchi at: kouchi@bs.aist-nara.ac.jp.

Contact Person: Kiyotaka Okada, Kyoto University

Email Address: kiyo@ok-lab.bot.kyoto-u.ac.jp

The Netherlands

The ARANED group, representing *Arabidopsis* researchers in The Netherlands, met in December 1994 in Wageningen and will meet again in January 1996 in Leiden. At the moment, 16 groups representing 1 company and 8 different universities and institutes are using *Arabidopsis* in their research programs. The largest groups are located at the Universities of Leiden, Utrecht, and Wageningen, in conjunction with The Dutch Center for Plant Breeding and Reproduction Research (CPRO-DLO). Numerous collaborations involving exchange of scientific materials and expertise are continuing as outlined in previous reports. Additional funding is being sought, both within the Netherlands and the European Union, for collaborative projects related to embryogenesis, plant hormones, photomorphogenesis, plant pathogen interactions, and ecophysiology.

Contact Person: Maarten Koornneef, Wageningen Agricultural University

Email Address: maarten.koornneef@botgen.el.wau.nl

New Zealand

New Zealand's Foundation for Research Science and Technology supports several *Arabidopsis* research programs. In Auckland, at the University and at Hort+Research, a T-DNA tagging project is underway and cloned genes and traits are being mapped to the *Arabidopsis* genome using the Lister and Dean Recombinant Inbred lines. These research projects are on the isolation and characterization of genes associated with flowering, carpel and silique development, resistance to viral pathogens, and response to aluminum stress. At AgResearch in Palmerston North, genes expressed in response to low phosphate levels are being characterized. Laboratories at other institutions in NZ are using cloned *Arabidopsis* genes to investigate flowering in kiwifruit, pine, ornamentals, and rye grass.

Contact Person: Jo Putterill, University of Auckland

Email Address: j.putterill@auckland.ac.nz

Republic of Korea

Approximately 15 laboratories in Korea are currently involved in *Arabidopsis* research or have expressed an interest in moving in this direction in the future. Subjects under study include mutational and molecular analyses of leaf senescence, biochemical and mutational analyses of light signal transduction pathways, regulation of wound-inducible genes, role of drought-induced protein kinases, protoplast culture, genes induced by heavy metals, salts, and other environmental stimuli, anther development, and gene expression in guard cells. Progress was also reported in the initiation of random antisense mutagenesis efforts involving cDNAs, an approach designed to complement the large-scale EST effort in *Arabidopsis*. In addition, more than 20 laboratories are involved in *Brassica* research in Korea and some of their efforts are related to *Arabidopsis* research. Projects include the generation of approximately 4,500 *Brassica* ESTs and comparative genome mapping between *Arabidopsis* and Chinese cabbage. These studies are funded primarily by individual grants from various government and private funding agencies. Laboratories involved in this work have agreed to establish a Korean *Arabidopsis* network in the coming year.

Contact Person: Hong Gil Nam, POSTECH

Email Address: nam@vision.postech.ac.kr

Spain

Arabidopsis has become an established experimental system in many Spanish laboratories of plant biology. Some research projects involving *Arabidopsis* deal with problems relevant to Spanish agriculture, such as

tolerance to salt, drought, and other types of biotic and abiotic stress. Other projects deal with developmental questions, ranging from leaf formation to the initiation of flowering, and their application to improving the quality and economic value of agricultural products. One laboratory at the CID/CSIC is already involved with the European project to sequence the *Arabidopsis* genome (ESSA) and others may join this effort in the future. Although there is no formal *Arabidopsis* research program in Spain, the National Program for Research and Development, recently approved for the 1996-1999 period, encourages within its Biotechnology Program the use of model species like *Arabidopsis* to facilitate the identification and characterization of genes of agricultural interest. Moreover, a proposal to create a Spanish collection of 10,000 T-DNA and Ds tagged lines was funded last summer by the Spanish Commission of Science and Technology. This collection is currently being developed at several laboratories within the Spanish *Arabidopsis* Network and will start to become available to other laboratories within this network by the spring of 1996. Eventually it is planned that the collection will become available to the rest of the *Arabidopsis* community through existing stock centers. The annual meeting of the Spanish *Arabidopsis* Network will be held in November, 1995 at the National Plant Molecular Biology meeting in Seville. New collaborations and joint projects are expected to be proposed at this meeting.

Contact Person: Jose Martinez Zapater, DPTO de Proteccion Vegetal

Email Address: zapater@cit.inia.es

United Kingdom

Research with *Arabidopsis* in the United Kingdom continues to gain momentum. Significant advances were reported over the past year in the areas of physical mapping, disease resistance, floral induction, hormone biology, and biochemical genetics. The Biotechnology and Biological Sciences Research Council (BBSRC) supports the majority of work on *Arabidopsis* in the UK. The other major source of funding is the European Commission. The BBSRC funds projects through its competitive grants program and through special funding initiatives. Ongoing initiatives include Plant Molecular Biology II (scheduled to end in 1997), Commitment and Determination, Plant and Animal Genome Analysis I and II, and Biochemistry of Metabolic Regulation in Plants II. At the present time, 78 grants involving work with *Arabidopsis* are being funded by the BBSRC. This past year has also seen the completion of the successful BBSRC postdoctoral fellowship program which supported two-year postdoctoral visits abroad followed by one year based in the UK. Justin Goodrich visited the Meyerowitz laboratory (linked with the Coupland lab at John Innes Centre, Norwich) and has since been awarded a Royal Society fellowship which he is taking to Edinburgh. Ottoline Leyser worked in the lab of Mark Estelle (Bloomington), linked with Ian Furner in Cambridge, and has now accepted a faculty position at the University of York. The Nottingham *Arabidopsis* Stock Centre continues to be well used by the scientific community throughout Europe. Current support from the BBSRC extends through April, 1997. The Stock Centre has also received a 3-year grant under the European Commission's Fourth Framework Biotechnology Programme to link with The Netherlands and France in the development and distribution of lines for insertional mutagenesis. Additional long-term funding for the Stock Centre is currently being sought.

Contact Person: Caroline Dean, John Innes Centre

Email Address: Arabidopsis@bbsrc.ac.uk

United States

Research with *Arabidopsis* remains firmly established in the United States, with active programs distributed throughout a wide range of academic, government, and corporate laboratories. The intensity of this research effort was evident at the Sixth International *Arabidopsis* Conference in Madison. This highly successful meeting was organized by a local steering committee headed by Rick Amasino at the University of Wisconsin, along with guidance from the North American *Arabidopsis* Science Steering Committee. The *Arabidopsis* Conference will be held again in Madison (June 26-30, 1997) following the meeting next year in Norwich (June 23-27, 1996). Federal grant support for research with *Arabidopsis* continued to increase with funding provided by a number of different agencies. One significant advance was the announcement of an interagency (NSF/DOE/USDA) request for proposals to support large-scale genomic sequencing in *Arabidopsis* (NSF publication 95-159). Sequencing efforts supported by this program are scheduled to begin later in the year. Federal funds continued to support a wide range of research projects headed by individual investigators while at the same time maintaining the essential *Arabidopsis* Biological Resource Center at Ohio State University and allowing the upgrade and transfer to Stanford University of the successful AAtDB database. Training of graduate students and postdoctoral fellows continued with support from fellowships programs at NSF, NIH, USDA, and DOE, the tri-agency (NSF/DOE/USDA) research training groups program, and individual research grants. The NSF postdoctoral research fellowship programs emphasize foreign research experience and the fellows have worked in laboratories in Australia, France, Germany, Japan, the Netherlands, Switzerland, and the United Kingdom. *Arabidopsis* plants are increasingly incorporated into a number of undergraduate laboratory experiences as well, offering hope that the scientific public will become increasingly more aware of the importance of basic research with model plants. This objective was boosted in recent months by the widespread media attention given to advances in our understanding of flower development reported by the laboratories of Detlef Weigel (Salk Institute) and Martin Yanofsky (University of California, San Diego). Positive stories describing this important research were carried by a number of US news organizations, including the Washington Post, New York Times, and National Public Radio.

Contact Person: David Meinke, Oklahoma State University

Email Address: meinke@osuunx.ucc.okstate.edu

ANALYSIS AND RECOMMENDATIONS

The original Long Range Plan for the Multinational Coordinated *Arabidopsis* Genome Project set forth a series of goals for the future. At the end of each year, progress toward these goals has been assessed, with new recommendations made for the future. The 1990 Report listed goals in six different areas, with target dates of 1, 2, 5, and 10 years. Now, in the fifth report, it is timely to assess how far we have come and what goals should be outlined for the future. Progress over the past five years is summarized in the table below and discussed in more detail in the text. This is followed by a list of recommendations for the coming year.

Overview of Progress Towards Project Goals

Program: Project Goal Outlined in Long-Range Plan	Target Year	Current Status
Genomics:		

Identify large numbers of genes by mutation	1	Completed
Construct YAC libraries with large inserts	1	Completed
Link ordered collections of YAC clones	2	Significant progress
Complete saturation mutagenesis programs	5	Significant progress
Begin cloning, analysis of identified genes	5	Ahead of schedule
Initiate large-scale genomic sequencing	5	Programs initiated
Complete sequence of entire genome	10	Target date extended
cDNA sequencing (*not in original plan)	*	Significant progress
Technology		
Support advances in genome technologies	5	Significant progress
Resources		
Establish multinational liaison committee	1	Completed
Establish centers for seed and DNA stocks	1	Highly successful
Electronic bulletin board and newsletter	1	Highly successful
Long-term support for resource centers	5-10	Currently pursuing
Informatics		
Form committee, draft informatics plan	1	Completed
Establish at least 1 electronic database	1	Completed
Easy access to map and sequence data	5	Completed
Design databases to support genome efforts	5	In progress
Training		
Fund short-term exchanges, short courses	1-10	Ongoing
Fund multinational *Arabidopsis* postdocs	1-10	Limited success
Symposia		
Support workshops and symposia	1-10	Ongoing

Genome Analysis

One-year goals in the area of genome analysis were to identify mutable genes and to make YAC libraries. Both of these goals have been achieved. Many laboratories have identified interesting mutations and participated in mapping efforts, and as a result, over 500 mapped genetic loci are known. Large-scale mutagenesis projects have been completed, and extensive screens for defects in embryogenesis, root and flower development, trichome differentiation, fatty acid and cell wall composition, amino acid biosynthesis, hormone response, and many other metabolic, physiological, and developmental processes have been performed. Several YAC libraries have been constructed and made available through the Ohio State Stock Center and the Koln DNA Stock Center, including the valuable addition this year of the CIC library with large inserts.

The two-year goal was to link ordered collections of YAC clones. This has yet to be completed, in part because technical obstacles were seriously underestimated, but the past year has seen a major breakthrough with the linking of chromosome #4 into just 4 contigs. The other chromosomes are nearing completion as well: chromosome #1 (42 contigs, 65% estimated coverage), chromosome #2 (4 contigs, 80% coverage), chromosome #3 (42 contigs, 60% coverage), and chromosome #5 (35 contigs, 85% coverage). An important goal for the coming two years must be the completion of the physical map.

Five-year goals included saturation mutagenesis (chemical and insertional) and initiation of large-scale genomic sequencing. Chemical mutagenesis programs are approaching saturation and are expanding to include screens for suppressors and enhancers. TDNA tagged lines representing thousands of insertions have been screened for mutant phenotypes and used to clone numerous genes. Projects designed to saturate the genome with transposon insertions are progressing as well. Largescale genomic sequencing has started in

Europe and is about to begin in the United States. Between these efforts, nearly 20 Mb of genomic sequence should be complete over the next three years. Another five-year goal was to begin cloning and analysis of genes identified by mutation. Progress here is ahead of schedule, with more than 30 genes identified by mutation already cloned. Continuing this effort is an important goal for the future. *Arabidopsis* leads the plant world in this type of work, and the genes being identified are an enormous resource for plant biologists. This work should continue at a high rate, and with full support of funding agencies.

Completion of the genomic sequence was originally a ten-year goal, with the explicit assumption that new technologies would emerge from the Human Genome Project. These new technologies have not appeared, and largescale sequencing is done today by the same methods as five years ago. In this part of the project, therefore, we are behind. A realistic new goal might be completion of 30% of the genomic sequence by the year 2000, and this assumes continued availability of funding. An important goal for the next five years is therefore to secure long-term funding from a number of different countries for largescale genomic sequencing projects so that the entire genome can be sequenced by the year 2004. In certain other respects, though, we are far ahead of projections. The original report did not envision the cDNA sequencing effort, and the current total of 22,500 cDNA sequences far exceeds even last year's projections.

Technology Development

Five-year goals were to support innovative technologies that addressed plantspecific problems and to apply methods from animals and fungi to *Arabidopsis* research. Both have been accomplished, although with such indeterminate goals there is no way to assess our degree of progress. Major breakthroughs have occurred in diverse areas ranging from high-efficiency transformation and insertional mutagenesis to the use of Green Fluorescent Protein as a reporter of gene expression. Clear advances have been made, and these efforts should continue as they are an essential component of scientific progress.

Biological Resource Centers

The goals in this area were to establish two stock centers with international liaison and advisory committees, and to operate an electronic bulletin board. Two stock centers have been established, one at Ohio State University in the U.S. and the other at the University of Nottingham in the U.K. Both are functioning extremely well, with overall progress monitored by advisory committees. The electronic bulletin board has grown to be a model for scientific communication and has become an established part of *Arabidopsis* laboratories around the world. An important goal for the future is to ensure continued support for stock center activities, which provide an essential foundation for *Arabidopsis* research.

Informatics

The goals here involved development of electronic databases to store and collate information on *Arabidopsis* research. Two databases with easy access to investigators worldwide were established several years ago, one associated with the stock center at Ohio State University (AIMS), and one providing extensive storage and display of genomic and reference information (AAtDB). Additional information is available on a variety of

WWW pages, including Weeds World, the electronic journal of *Arabidopsis* research, which is published by the Nottingham Stock Centre. The AAtDB database has recently been funded for major upgrading to the next generation of databases and moved from its previous location at Massachusetts General Hospital to Stanford University. This action follows recommendations outlined in the Year Three progress report. The current level of electronic integration of *Arabidopsis* information and transnational communication far exceeds that outlined in the original project.

Human Resource Development

The goals here were to support multinational *Arabidopsis* postdoctoral fellowships, shortterm exchanges, and short courses. Progress in this area has been mixed. For example, the Cold Spring Harbor Course on *Arabidopsis*, the yearly International *Arabidopsis* Meetings, and a number of transnational exchange programs have received considerable attention and support, but international postdoctoral fellowship for *Arabidopsis* research have not been established. We must continue to encourage these international fellowships so that students in many different parts of the world can remain active participants in plant genome research.

Recommendations for the Coming Year

We believe that progress as measured against the original goals has been very good, with multinational cooperation and scientific advances generally ahead of schedule except in large-scale genomic sequencing and international postdoctoral fellowships. Advances have continued at a rapid rate even as new goals such as cDNA sequencing programs have been added to the project. Specific goals for the coming year include:

- Establishment of large-scale genomic sequencing projects.
- Continued expansion and integration of physical, molecular, and genetic maps of *Arabidopsis*.
- Continued advances in the isolation and characterization of informative mutants and essential genes.
- Development of technologies that can readily test the functional significance of genomic sequences.
- Continued support for the stock centers and associated databases that provide vital services to the *Arabidopsis* community.
- Increased application of the information and resources gained through basic research with *Arabidopsis* to fundamental questions in biology and to research programs involving other plants.

Furthermore, the time may have arrived to draft a new set of goals to promote further advances in plant biology through the study of *Arabidopsis*. The Multinational Science Steering Committee therefore plans to assemble a group of scientists in the coming year to assist with drafting goals for future research efforts.