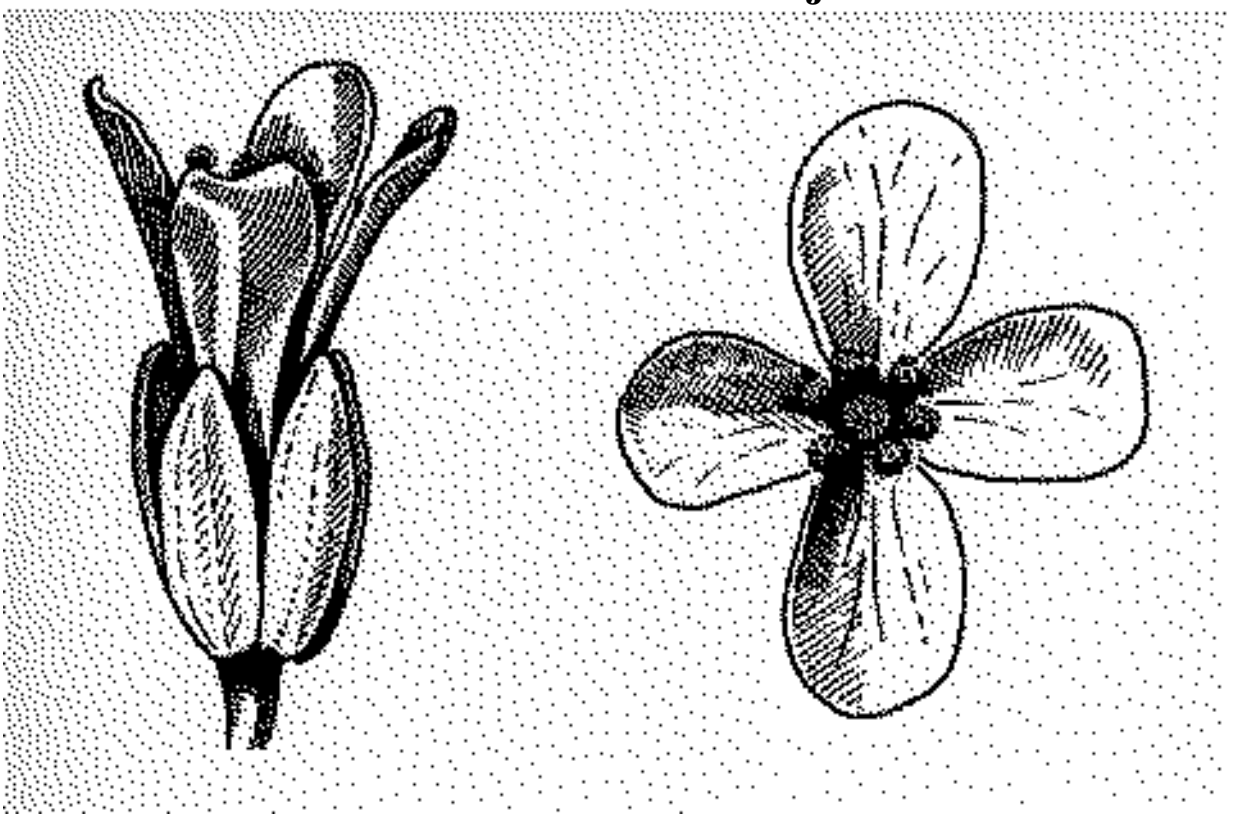
The Multinational Coordinated *Arabidopsis thaliana* Genome Research Project



Progress Report: Year Two

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Progress Report: Year Two

The Multinational Science Steering Committee

1992

PREFACE

In 1990, an *ad hoc* committee composed of nine scientists from the United States, Europe, Japan and Australia prepared a report entitled "Long-range Plan for the Multinational Coordinated *Arabidopsis thaliana* Genome Research Project". This publication (NSF 90-80) outlined a plan for international cooperation in understanding the structure and function of the genome of the higher plant *Arabidopsis thaliana*. The Committee assumed responsibility in six areas: (1) coordinate programmatic aspects of the *Arabidopsis* genome project; (2) communicate with the informatics and biological resource centers that were to be established; (3) monitor and summarize progress of scientific activities of participating laboratories; (4) serve as a liaison to the broader plant biology community; (5) identify needs and opportunities of the *Arabidopsis* research community and communicate these needs to the funding agencies of participating nations; and (6) periodically update the long-range plan.

This report of the Multinational Science Steering Committee is intended to summarize progress made during the past year and to foster communication among those scientists with a primary research interest in *Arabidopsis* as well as with other plant scientists and the general scientific community. We also identify areas of present need for the international group of funding agencies concerned with the project, and set forth a series of new goals for the coming year. A previous report, describing progress made during the first year of the project is available as publication NSF 91-60.

The information contained in this report was provided by members of the Multinational Science Steering Committee and by others acknowledged below[1]. Sources include both the published literature and less formal sources, including abstracts and presentations at symposia, communications of the *Arabidopsis* electronic newsgroup, and personal communications. The Committee outlined this report at Keystone, Colorado (USA) in April 1992. It is the nature of any general progress report, representing the work of hundreds of scientists worldwide, that some of the important experiments of the past year may have been overlooked or misrepresented. Therefore, we ask not only that our colleagues overlook such shortcomings but that they feel free to communicate their concerns and plans with their committee representatives, so that future reports will be as accurate as possible.

The Multinational Science Steering Committee
Chairman: Marc van Montagu
Members: Caroline Dean, Richard Flavell, Howard Goodman, Maarten Koornneef, Elliot Meyerowitz, Jim Peacock, Yoshiro Shimura, Chris Somerville

August 1992

I. Progress of the Previous Year

Because *Arabidopsis* is now widely used as an experimental organism, it has become

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[1] Among those to whom we are indebted for information used in the initial draft report, and for the subsequent revisions are M. Anderson, F. Ausubel, A. Bent, M. Boguski, M. Caboche, M. Cherry, J. Chory, I. Crute, J. Dangl, M. Delseny, D. Flanders, R. Gardner, R. Hangarter, E. Holub, D. Kristofferson, D. Meinke, K. Okada, S. Pramanik, M. Schena, R. Scholl, P. Scolnick, P. Sijmons, S. Somerville, and R.F. Whittier.

increasingly difficult to formulate a concise description of progress. The growth of information about *Arabidopsis*, in particular, and plant biology, in general, is exploding. Much of the general progress in this discipline is directly attributable to the fact that *Arabidopsis* has many attractive features as an experimental organism. In addition, a substantial proportion of the visible progress is attributable to the advantages of having a large number of scientists working on a single organism where information, methods and materials can be freely exchanged. Several of the initial objectives of the *Arabidopsis* genome project, which were designed to facilitate these advantages, have been implemented during the past year and appear to have had the desired effects. The results of these initiatives, and the projected effects of new initiatives are summarized in the following sections under the major topics of genome analysis, technology development, resource centers, informatics, human resource development and symposia. In addition, various organized national research projects coordinated as the Multinational *Arabidopsis thaliana* Genome Research project, are described.

1. Genome Analysis

In the original conception of the *Arabidopsis* Genome Research Project, a broad approach to the dissection of the genome was envisioned. The rationale was that in order to interpret the coding capacity, it was most desirable to characterize genes by all available criteria. These included mutational analysis, the mapping of new mutations and genes, the mapping of RFLP markers and the cloning and sequencing of genes on the basis of functional criteria, as well as the eventual objective of obtaining the entire nucleotide sequence of the genome. Progress on these fronts is summarized below under three headings: maps, mutations, and new genes cloned and sequenced.

A. Maps

One of several promising genetic methods for the isolation of new genes is map based cloning in which a gene is isolated solely on the basis of genetic map position. If a sufficiently large number of RFLP markers were mapped, it would be possible to isolate virtually any gene for which a mutation was known by probing a suitable genomic library with the RFLP markers which closely flank the gene of interest, and isolating clones which carry both of the flanking markers. It is, therefore, of substantial importance to have a high density, accurate genetic map available for *Arabidopsis*. An international collaboration to integrate the two RFLP maps with a total of approximately 300 markers, and the map of approximately 120 visible markers is nearing completion. In addition, a new map based on randomly amplified polymorphic DNA (RAPD) markers and recombinant inbred (RI) lines was published (Reiter et al., Proc. Natl. Acad. Sci. USA 89,1477, 1992). This map contained 252 new markers which were mapped relative to 60 previously mapped RFLP markers. The RI lines have been deposited with the Nottingham and Ohio Resource Centers. Because they have been scored for a large number of genetic markers, the availability of these lines will greatly facilitate the high resolution genetic mapping of cloned genes.

Another project to add an additional 140 RFLP markers is nearing completion in Japan (Mitsui Plant Biotechnology Research Institute, Tsukuba). These markers are being mapped using another set of RI lines produced and characterized by C. Lister and C. Dean (Norwich, U.K.) so there should be no impediment to the integration of these additional markers with

existing maps. Hybridization of 30 markers from each of the two previously published maps onto these RI lines has also improved the integration of the two maps.

Progress towards completion of a physical map continues on several fronts. A collaborative effort by several U.S. laboratories and a British group has identified and mapped yeast artificial chromosomes (YACs) covering approximately 33,000 kb or about one third of the genome (Hwang et al., and Schmidt et al., Plant J. 1, 367 (1991)). The YAC libraries used in these experiments are also being used to link up the approximately 750 cosmid contigs representing the entire genome, which were produced by H. Goodman, B. Hauge and collaborators (Boston, MA). A systematic effort on genome linking using YAC to contig hybridization is underway in H. Goodman's laboratory (Boston, MA). To date, Goodman and his colleagues have completed approximately 2,500 hybridizations (approximately four genome equivalents of YAC clones) to the gridded cosmid filters containing the 1,920 cosmids and approximately 500 hybridizations to the gridded cosmid filters with 480 cosmids. They have written computer programs to transform the data into a form suitable for orienting with the contig map, and are in the early stages of comparing the hybridization linkages with the established contig map. The complete collection of 1,920 cosmids used on the filter grids has been archived with the intention of making it available to the community.

As noted below, the widespread use of the YAC libraries for chromosome walking has resulted in the construction of maps covering more than 1 million bp for at least four regions of the genome. However, because of the presence of chimeric YACs and the frequent occurrence of low copy number repetitive sequences in the *Arabidopsis* genome, constructing a physical map by using ends of the YACs as hybridization probes has progressed slowly. Because of increased interest in large-scale sequencing of *Arabidopsis* cDNAs (see below), the most economical option may be to link up the YACs by hybridizing a large number of partially sequenced cDNA clones to the YAC libraries. This would provide useful information about the cDNA clone and would frequently provide information about overlap of YACs. Progress toward a complete YAC map should also be greatly facilitated by the completion of a new YAC library by J. Ecker and colleagues (Philadelphia, PA) which reportedly has significantly larger inserts than previous libraries.

Several important initiatives, such as large-scale cDNA sequencing, and the development of physical maps, are predicated on the concept that these methods will eventually intersect with the results of research which is driven by specific biologically based questions. The probable mechanism of integration is at the level of the genetic map. Thus, the utility of these initiatives will ultimately depend upon the richness of the information anchored by mapped mutations. The number of published newly mapped mutations increased by approximately 20% during the past year. As an indication that significant expansion of the genetic map is pending, work is currently underway in the Meinke laboratory (Stillwater, OK) to map 100 new embryo defective mutations.

B. New Mutations Characterized

If the complete sequence of the *Arabidopsis* genome were available today, it would not be possible to deduce the function of most of the genes because a relatively small proportion of the genome has, as yet, been marked by informative mutations. Therefore, one of the highest priorities for the genome project remains the development of an exhaustive collection of characterized and mapped mutations. Although it has not been possible to catalog all of the newly described mutations, progress on this front continues at an explosive pace. Indeed, one of

the plant biology journals (Plant J.) has instituted a new section for short reports of new mutations in a manner analogous to the short reports of new gene sequences which were used before the advent of widespread and facile access to the nucleotide sequence databases. Unfortunately, there appears to be a substantial lag between the isolation of a new mutation and its appearance on the linkage map. It is hoped that the development of several new databases (see below) will facilitate and accelerate the dissemination of this important information.

An example of recent progress in the identification of new mutations during the past year has been the maturation of a broadly based thrust to develop well-defined host/pathogen systems of *Arabidopsis* and the characterization of genetic variation for resistance or susceptibility. In spite of the importance of disease to crop productivity, almost nothing is known about the molecular mechanisms which specifically mediate the plant response to pathogens. Thus, the rapid progress towards isolation of several disease resistance loci in *Arabidopsis* promises to have direct practical applications in agriculture.

Pseudomonas syringae, *P. cichorii*, and *Xanthomonas campestris* strains that develop compatible or incompatible interactions in *Arabidopsis* have been identified. At least two cloned bacterial avirulence (avr) genes, avrRpt2 and avrRpm1, elicit the hypersensitive necrosis response in an ecotype specific manner in *Arabidopsis*. At least four mutant alleles of a nuclear locus in *Arabidopsis* fail to give a hypersensitive response when infiltrated with *P. syringae* carrying the avrRpt2 gene, and an allele of a nuclear locus, found as a naturally occurring variation in *Arabidopsis* ecotypes from the Kranz collection, confers resistance to avrRpm1. Efforts are now underway in several laboratories to clone these putative resistance genes using chromosome walking techniques. At a recent meeting in Köln (Germany) several laboratories reported that they had identified RFLP markers within 1 to 2 cM of disease resistance genes and the laboratory of J. Dangl (Köln, Germany) reported the isolation of a YAC fragment which appears to carry the resistance gene RPM1.

Substantial progress has also been made in defining genes which confer susceptibility or resistance to two obligate fungal biotrophs *Albugo candida* and *Peronospora parasitica*. Screening of *Arabidopsis* accessions from the public strain collections identified lines which were resistant or susceptible to *Peronospora*, *Albugo* and the powdery mildew pathogen *Erysiphe cruciferum*. A hypothetical model involving eight resistance loci has been proposed to explain differential interactions between six accessions and six isolates of *Peronospora*. One of the loci has been mapped near *gl1* on chromosome 3. Two other loci, *RPP2* and *RPP4*, have been located near to each other on chromosome 4. In addition, thirteen *Arabidopsis* accessions have been found on which sporulation by *Albugo* does not occur or is delayed. The first resistance locus (*RAC1*) is currently being mapped.

A variety of viruses are infectious in *Arabidopsis*, including cauliflower mosaic virus and turnip crinkle mosaic virus, and at least one virus-resistant ecotype has been identified. *Arabidopsis* has also been shown to be a good host for several sedentary root nematodes including the cyst-knot nematode *Heterodera schachtii* and the root-knot nematode *Meloidogyne incognita*. One advantage of *Arabidopsis* for the study of nematode-root interactions is that the roots are sufficiently transparent to observe the entire life cycle of the nematodes using video-enhanced contrast light microscopy. This has provided a unique opportunity to observe the infection cycle in intact living tissue for the first time.

In addition to the direct analysis of disease resistance mechanisms involving major genes, the rapidly expanding genetic resources in *Arabidopsis* provide unique opportunities to examine the role of variation in other aspects of plant biology. For instance, ethylene has been

hypothesized to play a role both in disease resistance and in disease susceptibility. This was tested using isogenic virulent and avirulent bacterial pathogens, and mutants of *Arabidopsis thaliana* altered in ethylene physiology. Ethylene-insensitive *ein1* and *ein2* mutants of *Arabidopsis* were resistant to *P. syringae* pv. tomato made avirulent by addition of the cloned avirulence genes avrRpt2, avrRpm1, or avrB, suggesting that ethylene is not required for active resistance against avirulent bacteria. As another example, the structure of an *Arabidopsis* phytoalexin (camalexin) was determined and mutants deficient in the accumulation of camalexin were isolated. The availability of mutants should facilitate a direct and unequivocal test of the role of phytoalexins in disease resistance to a broad spectrum of bacterial and fungal pathogens. In addition, mutants have been isolated that show accelerated cell death symptoms (*acd* mutants) when infected with bacterial or fungal pathogens.

C. Genes Cloned and Sequenced

The most important method for the isolation of new genes in *Arabidopsis* has become the use of T-DNA tagging developed by K. Feldmann and D. Marks. At present, there are approximately 13,000 lines in the collections maintained by DuPont and the two *Arabidopsis* Resource Centers, each of which has a randomly inserted T-DNA. Approximately 30% of the mutations observed in these lines are due to the insertion of T-DNA into a gene which can then be readily cloned. The relative ease with which T-DNA tagged genes can be cloned has led to rapid progress in understanding several previously intractable problems. For instance, T-DNA tagging and related methods have been exploited with spectacular success to isolate a number of genes involved in floral morphogenesis. During the past year at least 3 new genes involved in flower development (APETALA3, LEAFY, PISTILLATA) have been isolated and a large group of new enhancers, suppressors, and potentially tagged new flower mutations have been isolated by E. Meyerowitz and collaborators (Pasadena, CA). In parallel with the isolation of genes by genetic methods, a relatively large number of genes of already known function have been isolated and characterized. For instance, because of the rapidly expanding interest in pathogenesis in *Arabidopsis*, a variety of defense-related genes have been isolated for use in monitoring reactions to pathogens. These include PAL1 (phenylalanine ammonia lyase), CHS (chalcone synthase), BGL1, BGL2, BGL3 (ß-1,3-glucanases), GST1 (glutathione-S-transferase), SOD1 (superoxide dismutase), and LOX1 (lipoxygenase). Several calmodulin-like proteins, chitinase and HMG CoA reductase genes have been cloned. The number of cloned and sequenced genes in the nucleic acid databases currently totals almost 300.

D. Large Scale Sequencing

The EC (European Community) is planning to fund a major initiative on sequencing *Arabidopsis*. The working title is ESSA - European Scientists Sequencing *Arabidopsis*. Some funding will be allocated to small scale sequencing in which well-characterized regions of the genome will be sequenced at a rate of 25 kb/year. It is expected that grant proposals to sequence cDNA clones on a similar scale will also be solicited. A small number of grants will be awarded to undertake medium-scale sequencing of contiguous regions of approximately 1,000,000 bp per year. It is estimated that there are currently three European laboratories which possess the infrastructure to mount medium scale sequencing efforts. A medium-scale sequencing project is

also underway in the laboratory of H. Goodman (Boston, MA). In preliminary feasibility studies, 35 kb of contiguous sequence has been completed.

Large-scale cDNA sequencing has recently begun in France and the USA. The French cDNA sequencing initiative is supported by CNRS. This project, in which 8 groups are participating, is part of the French Government's initiative on Genome Projects. Each group has prepared its own cDNA library corresponding to the biological problem it is interested in, and has started to sequence, from both ends, cDNA clones selected either at random or after differential screening. Nine months after the start of this program, approximately 600 clones have been partially sequenced. Although limited, the available sequence information has allowed the identification of slightly more than 200 genes by comparison with sequences already in databanks. About half of them had not been identified previously in plants. The initial goal is to partially sequence 3-4,000 clones. The general idea is to use these tagged sequences to establish a transcription map of the *Arabidopsis* genome and to facilitate the genome sequencing projects by providing the corresponding cDNA clones. These efforts will be integrated with the EC BIOTECH program mentioned above and, through the use of common database (AAtDB), with the other programs in the world. The coordinator of the program is Bernard Lescure (Laboratoire de Biologie Moléculaire des Intéractions Plantes-Microrganismes, INRA-CNRS B.P. 27, 31326 Castanet-Tolosan).

In the U.S., a cDNA sequencing laboratory has also been established at Michigan State University with support from the U.S. Department of Energy Biological Energy Research Program and the State of Michigan. The sequencing project was organized as a joint project by ten laboratories at MSU and is managed by T. Newman. Sequencing reactions are performed by an ABI Catalyst robot and resolved on ABI 373A Sequenators. Sequencing has begun on random clones from a cDNA library representing roots, shoots, leaves, and etiolated seedlings. The MSU project anticipates achieving a throughput rate of 200 partial cDNA sequences (approximately 500 bp each) per week by the end of the year. The sequences are being deposited on a daily basis in dbEST, a new public access database for expressed sequence tag (EST) sequences operated by the National Center for Biotechnology, National Library of Medicine. This database utilizes preprocessing and filtering tools that make EST analysis more efficient and effective than the tools available in the larger databases (eg. GenBank). For more information about dbEST, contact Mark Boguski at boguski@ncbi.nlm.nih.gov (Internet) or 301-496-2475 (phone). In addition, sequences will be analyzed for the presence of known motifs and homology to known proteins by E. Retzel and colleagues at the University of Minnesota. Results of these analyses will be deposited in the AIMS database.

2. Technology Development

A. Transformation Methods

During the past year the number of laboratories in which transformation has become routine has greatly expanded due to the identification of several races of *Arabidopsis* which respond well to improved transformation protocols. In addition, there have been several reports that a method of *Agrobacterium*-mediated transformation reported several years ago by Hong-Gil Nam (Korea) has been implemented in several laboratories. This method, which is an adaptation of the method used by Feldmann to generate T-DNA inserts, involves inoculating

decapitated plants with *A. tumefaciens*. Approximately one plant in five reportedly produces at least one transformed progeny from such an inoculation. The method may have the potential advantage of being race-insensitive and requiring substantially less effort than tissue-culture based methods.

Important progress has been made during the past year in methods for large-scale transformation of protoplasts and regeneration of transformed plants. The development of these capabilities represents tangible progress toward the much needed methods for homologous gene replacement and, possibly, the development of PACs (plant artificial chromosomes).

B. New Gene Cloning Methods

a. Chromosome walking

One of the important advantages of *Arabidopsis* is the small genome size and the relatively low abundance of interspersed repetitive DNA. This makes it feasible to undertake gene isolation by chromosome walking from flanking RFLP markers. There are currently approximately 40 chromosome walking efforts underway worldwide. Several of these have recently resulted in the isolation of functional genes which have complemented the mutations used to mark the genes. In one instance, V. Arondel (East Lansing, MI) and collaborators cloned a gene encoding an intractable enzyme in lipid metabolism by mapping a mutation affecting membrane lipid composition and using the flanking RFLPs to isolate YACs covering the mutation. The identified YACs were used to screen a cDNA library, and the gene identified by transforming cDNA clones into the mutant in order to identify a clone which complemented the mutation. In another instance, M. Estelle and collaborators (Bloomington, IN) isolated a gene involved in mediating the response to exogenous auxin by identifying YACs covering the *axr1* mutation, then using a DNA rearrangement induced by the mutation to identify the region of YAC DNA containing the gene. The isolation of these genes is expected to be followed in the near future by the isolation of genes involved in pathogenesis, phytohormone and phytochrome responsiveness, and regulation of flowering among other things. At least four walks have covered regions of the genome of between 1000 and 1300 kb. Thus, although most scientists involved in the walking efforts have found the approach to be expensive and demanding in many respects, the fact that it is possible at all represents important progress. It is no longer necessary to abandon an interesting mutation because of the lack of a method for isolating the gene. If the mutation is sufficiently interesting, it can be cloned.

Progress on several fronts promises to facilitate the cloning of genes by map-based cloning methods. Because of the participation of a relatively large number of laboratories in chromosome walking efforts, the genetic maps in the regions of the walks have been significantly refined and the YACs covering these regions defined. This process has already resulted in the identification of YACs covering more than one third of the genome. As this process continues, it will be increasingly possible to identify the YACs covering a region of the genome by reference to a database. In addition, the recent development by J. Ecker's laboratory (Philadelphia, PA) of a YAC library with significantly increased insert size will greatly facilitate the development of an overlapping YAC library of the entire genome.

b. Insertional mutagenesis

The most efficient method of cloning genes has been the use of T-DNA tagging. Mutants from a major collection of lines carrying T-DNA inserts, which is maintained by DuPont Central Research (Wilmington, DE), was distributed to 135 laboratories around the world during the past year. The pooled transformed lines and individual visible mutants, generated by *Agrobacterium* seed transformation in the Feldmann laboratory, are being distributed through the Nottingham and Ohio stock centers. A total of 5,260 transformants, including 460 transformants generated at Zoecon in 1986-87, and 4,800 generated at the University of Arizona in 1991, are available at present. The transformed families have been subjected to a preliminary screen. Most of the mutant families identified in these preliminary screens have been or are being sent to the two stock centers.

Approximately 800 putative mutants were identified in the preliminary screen. These include:

>60 flower mutants
>150 embryo-defective mutants
~150 size variants
>50 seedling-lethals
>100 pigment mutants
>100 other various morphological mutants.

The mutants listed above are currently available from the stock center or will be in the next month.

A great deal of work has been involved in creating an active transposon for *Arabidopsis*. The first products of this effort have been realized by the isolation of several Ds-caused mutants by the groups of C. Dean and G. Coupland (Norwich, U.K.). Also, in the process of characterizing a mutation which arose in one of the Ac lines generated by the Dean group, a new transposon was isolated in a spontaneous mutant of the *chl1* locus. Work in the laboratory of N. Crawford (La Jolla, CA) indicates that this transposon is apparently active and may be useful as an endogenous tagging system.

3. Biological Resource Centers

One of the initial objectives of the genome project was realized this year with the opening of the *Arabidopsis* Biological Resource Center at Ohio State University (ABRC), in addition to the previously established Nottingham *Arabidopsis* Stock Center (NASC) at the University of Nottingham, U.K., and the DNA Resource Center in Köln, Germany. The Nottingham Center was established under the direction of B. Mulligan and M. Anderson with five years of funding from both the U.K. Agricultural and Food Research Council and the EC BRIDGE program. The OSU Center, which is directed by R. Scholl and K. Davis, was established with a five year grant from the National Science Foundation. The Köln Center directed by J. Dangl is supported by EC-BRIDGE program. NASC and ABRC are operated as parallel collections which closely coordinate the maintenance and collecting of plant materials. As a general rule, it is expected that ABRC will service North America and NASC will service the rest of the world.

The primary functions of these resource centers are to acquire, preserve and distribute seed and DNA stocks for use by the research community. ABRC also collects and disseminates

information concerning *Arabidopsis* and will provide a fully computerized stock information/ordering system. In this regard, the Center is cooperating with S. Pramanik of Michigan State University to develop a comprehensive database for *Arabidopsis*, AIMS (*Arabidopsis* Information Management System). The Center, in addition to the co-principal investigators, employs four technical staff.

The two seed stock collections currently maintain the Koornneef mutant and marker line collection, a collection of approximately 400 T-DNA insertion lines from C. Koncz (Köln, Germany), a collection of 5,000 T-DNA tagged lines from K. Feldmann (Tucson, AZ), the collection of ecotypes and mutants previously maintained by A. Kranz (Frankfurt-am-Main, Germany), and many mutant and marker lines contributed by other laboratories. In addition, the Centers distribute 100 recombinant inbred lines contributed by P. Scolnik (Wilmington, DE). Another 300 recombinant inbred lines from another cross will be contributed by C. Dean (Norwich, U.K.) in October, 1992. ABRC is working with G. Redei (Columbia, MO) to transfer his collection to the Ohio Center.

During 1991, the Nottingham Center distributed 791 seed lines and during the first five months of 1992, more than 3,000 packets of seed were distributed to various locations around the world. Since the ABRC began accepting orders on April 20, 1992 more than 12,000 seed stocks had been distributed by August 1, 1992. Major contributions to these numbers are represented by the Du Pont recombinant inbred lines and the Feldmann *Agrobacterium* mutants and transformant pools. In addition, large numbers of tester lines, individual mutants and T-DNA insertion mutants have also been shipped. It is obvious that there exists a large demand for these resources.

Catalogs of the collections are available from the resource centers upon request. In addition, as noted above, the AIMS database currently under development will be utilized to keep the ABRC stock information current, for direct ordering of stocks and as a general information resource on *Arabidopsis*.

Requests for stocks from the stock centers can be submitted by any of several means: Specifically, a completed order form can be mailed, faxed or e-mailed to the Center. The addresses for ordering are:

Biological Resource Center at Ohio State, 1735 Neil Avenue, Columbus, OH 43210, USA fax: 614-292-0603
telephone 614-292-9371
e-mail, seeds: seeds@genesys.cps.msu.edu (for seed orders)
e-mail, DNA: dna@genesys.cps.msu.edu (for DNA orders)
(NOTE: For e-mail orders, type "STOCK ORDER" in the subject line.)
The ABRC Stock list is deposited in the directory, Public/BIOSCI/ARABIDOPSIS. The file name is ABRC_stock_lists and it can be recovered by anonymous FTP from genbank.bio.net.

For the Nottingham Center, contact:

Dr. M. Anderson, NASC, Department of Life Science, University of Nottingham, University Park, Nottingham, NG7 2RD, UK
Telephone: 44-602-791216
Fax: 44-602-513251
E-mail: PLZMLH@VAX.CCC.NOTTINGHAM.AC.UK

In addition to seed stocks, ABRC also maintains samples of plasmid, cosmid and YAC clones. These include the mapped RFLP phage (from E. Meyerowitz) and cosmids (H. Goodman), the YAC libraries produced by E. Grill (E. Lansing, MI) and E. Ward (Research Triangle, NC) and individual genes. The Center accepts deposits of any cloned and characterized *Arabidopsis* gene. In the three months of operation, ABRC has distributed 517 stocks of cosmid and phage clones and nine complete YAC libraries have been distributed. Anticipated DNA acquisitions include more RFLP clones, genomic and cDNA libraries, and individual clones. The DNA Resource Center maintained by J. Dangl at the Max Planck-Institut für Züchtungsforschung in Köln (Germany) also distributes cDNA and genomic libraries which have been deposited by members of the community. The Köln Center filled more than 30 requests for RFLP probes, YAC libraries and phage libraries in 1991 and 16 in 1992, so far. For information about the Köln collection contact J. Dangl at DANGL@VAX.MPIZ-KOELN.MPG.DBP.DE (E-mail) or 49-221-5062-613 (Fax).

4. Informatics

Because of the explosion of information about *Arabidopsis*, it has become essential to have mechanisms to collect and organize the mass of new information. A tangible step forward was implemented with the recent release of the *Arabidopsis* genome database, AAtDB (See Appendix I). AAtDB (An *Arabidopsis thaliana* Data Base) uses the excellent ACeDB software written by Richard Durbin (MCR-LMB, U.K.) and Jean Thierry-Mieg (CNRS, France). AAtDB is funded by the U. S. Department of Agriculture Plant Genome Research Program through the National Agricultural Library and is maintained by H. Goodman and colleagues at the Massachusetts General Hospital and Harvard University in Boston. AAtDB is available without charge via Internet network transfer.

The ACeDB software allows the user to browse information by simply pointing and clicking with the workstation mouse. A powerful query facility is also available.

Currently AAtDB contains:

- The Hauge/Goodman cosmid/YAC physical map including >14,000 cosmid clones.
- Genetic markers, both RFLP and classical markers.
- Unified Genetic Map, including both the Goodman and Meyerowitz RFLP markers and classical genetic markers.
- Primary F2 mapping database from the Goodman and Meyerowitz RFLP mapping projects.
- Primary two point recombination data from M. Koornneef.
- A strain catalog including all strains and clones available from the Nottingham Stock Centre and the ABRC at Ohio State University.
- Bibliographic citations from 1964 to present, currently numbering over 2,700.
- List of *Arabidopsis* researchers including mail address, phone number, FAX number and electronic mail address. Currently information on over 500 colleagues is included.
- Green Book. The Green Book by Meyerowitz and Pruitt has been updated and integrated into many parts of the database, including phenotype and allele descriptions.
- All DNA sequences from GenBank, currently there are over 300 sequences.
- BLASTX defined peptide sequence homologies.

- REBASE restriction enzyme database maintained by R. Roberts.
- Graphical displays of all Genetic Maps, Physical Maps, and DNA Sequence features and homologies.
- Scanned images of RFLP autoradiograms and photographs of mutant phenotypes.

The database presents the information in separate windows. There are many paths to any piece of information allowing the user to easily navigate the connections between the various types of information. As information about the *Arabidopsis* genome is expanded, AAtDB will be extended and enhanced via periodic updates.

The database currently requires a Unix workstation running X-Windows. Versions of the ACeDB database software are available for Sun Microsystems SPARCstations, Digital Equipment's DECstation, Silicon Graphics Iris series and NeXT workstations. A printed manual "An Introduction to ACeDB: For AAtDB--*A. thaliana* database" is available on request from the MGH group. A MacIntosh version of the ACeDB software is under development. A public release version is expected within six months. For more information contact Mike Cherry or Sam Cartinhour at FAX number 617-726-6893 or via electronic mail at curator@frodo.mgh.harvard.edu. By mail, contact Sam Cartinhour, J. Michael Cherry or Howard M. Goodman, Department of Molecular Biology, Massachusetts General Hospital and Department of Genetics, Harvard Medical School, Boston, MA 02114, USA.

A second database which will be available on-line via the Internet has been developed by a collaboration between the OSU Resource Center and the laboratory of S. Pramanik (E. Lansing, MI). The database, which is written in the very powerful Sybase language, will be directly accessible by FTP via the Internet from any computer platform (i.e., Mac, PC or workstation). The first release, which is currently being tested at several sites, will contain the information on stocks in the resource centers and will permit ordering of seeds or DNA via electronic media (See Appendix II). The full system, which should be implemented around the end of 1992, will have complete genetic, phenotypic and other important data for germplasm, relevant information for all clones, consensus genetic and physical maps and mapping data, and bibliographical information. The feature of this database that distinguishes it from other species genome databases is the attempt to incorporate detailed, atomized phenotypic information and to allow complex searching involving phenotype. The MGH and MSU groups collaborate and coordinate their activities in order to serve efficiently the *Arabidopsis* research community.

In order that the cDNA sequences produced by any large-scale sequencing efforts be of the broadest possible utility, it is essential that they be deposited in a public access database. However, partial cDNA sequence data is qualitatively different from traditional types of entries in the general purpose sequence libraries. For instance, because the coding sequence and reading frame of a partial cDNA sequence is not known for an EST (expressed sequence tag) in advance, searching an EST collection for deduced amino acid sequence homology requires translating all six frames with an amino acid query sequence, a function which is not routinely available on the other databases. In addition, the map location of an EST, and not its relationship to known sequences, may be the information of interest and this information is not explicitly retrievable from the general purpose sequence collections. Because of the parallel need for such a database structure in other genome projects, the National Center for Biotechnology Information (NCBI) of the National Library of Medicine / the National Institutes of Health, has created a specialized database called "dbEST" (data base of Expressed Sequence Tags). The *Arabidopsis* community has been invited to deposit partial cDNA sequences in this database. The large-scale sequencing effort underway at Michigan State University will also deposit all sequences in this database.

For more information contact Mark Boguski at:

National Center for Biotechnology Information, National Library of Medicine, Bldg, 38A, NIH,
8600 Rockville Pike, Bethesda, MD 20894, USA
Phone: 301-496-2475
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The EC BRIDGE project has established a committee to evaluate the needs and conditions for operation of an European node to collect, analyze, disseminate and store genome data. The members of the committee are M. Bevan (chair), J. Giraudat, D. Inze, M. Zabeau, and J. Dangl. Following several meetings, including a joint EC-US workshop in January 1992, a study report has been prepared which outlines the proposed structure of an EC *Arabidopsis* database.

5. Communication

Since the inception of the electronic *Arabidopsis* newsgroup two years ago, most of the laboratories working on *Arabidopsis*, and many scientists with a primary research interest in other organisms, have subscribed to the newsgroup. The newsgroup is distributed worldwide through both USENET news (under the name of bionet.genome.arabidopsis) and through e-mail. There are currently 402 e-mail subscribers, 299 subscribed through the U.S. BIOSCI distribution center at GenBank/IntelliGenetics and 103 subscribed through the BIOSCI center at SERC Daresbury Laboratory in the U.K. While it is difficult to estimate precisely the number of people who access the group via USENET news, a survey on another BIOSCI newsgroup about a year ago indicated that almost 50% of the readership used USENET. This suggests that the total newsgroup readership could be approximately 800 people at sites around the globe. There have been 344 postings to the newsgroup during the
period 1 January 1992 through 27 July 1992 (209 days) for a usage rate of 1.65 messages per day. This compares to a total of 264 messages posted during 1991 (0.94 per day over the period 3/26/91 to 12/31/92). The usage rate is up by 76% compared to last year. While overall 1992 statistics for BIOSCI remain to be compiled, in 1991 the *Arabidopsis* newsgroup was the seventh most active group. The most active newsgroup, BIONEWS, averaged 3.78 messages per day in 1991 compared to 0.94 for *Arabidopsis*. The current year has seen a continuing growth in the use of most BIOSCI forums as more biologists become familiar with electronic communications. An archive of postings is maintained for anonymous FTP at genbank.bio.net in the directory pub/BIOSCI/. A listing of the available files follows:

```
-rwxrwxr-x  1 kristoff   275510 Jan  2  1992 1991 (archive for 1991)
-rwxrwxr-x  1 kristoff   594778 Jul 27 13:46 1992 (archive for 1992)
-rwxrwxr-x  1 kristoff    51562 Jul 10 14:25 ABRC_stock_lists
-rwxrwxr-x  1 kristoff    70170 Apr  6 11:58 arab-gen.list (subscribers as of early 1992)
-rwxrwxr-x  1 kristoff    34932 Apr  6 11:58 arab-gen.list.pt1
-rwxrwxr-x  1 kristoff    35238 Feb  4 15:46 arab-gen.list.pt2
```

Postings are also indexed in the general "biosci.src" WAIS source on the computer genbank.bio.net. WAIS software indexes all text in every BIOSCI newsgroup posting and

allows users on the Internet to search for any text string and then retrieve messages bearing the specified text.

In order to subscribe to the newsgroup send a message requesting information on how to subscribe to biosci@net.bio.net.

A useful and enjoyable source of information for those working on *Arabidopsis* has been the quarterly newsletter sponsored by the British *Arabidopsis* group and edited by David Flanders (Norwich, U.K.). Unfortunately, because of the limited funding available to publish this lively and interesting newsletter, its circulation has been limited. However, it is expected that a large-circulation newsletter, edited by several people worldwide, will appear in the forthcoming year and will continue in the same vein as the British newsletter (which was variously known as Arabadabadopis, Arabidian Notes, Thale and Cress, Arabido and several other pseudonyms).

6. Workshops and Symposia

An important mechanism for stimulating communication among plant biologists has been the convening of a number of specialized and general meetings focused on the use of *Arabidopsis*. An international ARAPANET (*Arabidopsis* Pathogenesis Network) workshop on pathogenesis was organized by Jeff Dangl in Köln, Germany. The workshop was attended by approximately 50 people, 20 of whom were working with bacteria, fungi, viruses, and nematodes that infect *Arabidopsis*.

A workshop to discuss genome research and database issues was held at Massachusetts General Hospital (Boston, MA) in January 1992. Approximately 20 representatives of the efforts underway in the U.S. and five European nations participated in a roundtable discussion which was primarily focused on database issues associated with large-scale sequencing efforts. A major result of the meeting was broad consensus that large-scale cDNA sequencing projects must be organized in such a way that the community at large has rapid access to the information via electronic databases. The broad consensus on this issue obviated discussion about the problems created by the practice of seeking patents for partial cDNA sequences which was initiated by the National Institutes of Health. There was unanimous agreement among the participants of the Boston workshop that no attempt should be made to patent partial *Arabidopsis* cDNA sequences.

The Fourth International Conference on *Arabidopsis* Research, which was attended by approximately 450 participants, was held in Vienna in June 1990. The 5th International conference is scheduled to be held at Ohio State University in 1993. The tentative dates are August 19 to 22. The organizing committee is Keith Davis, Ken Feldmann, Randy Scholl, and Roger Hangarter. For information, contact:

Roger P. Hangarter, Department of Plant Biology, Ohio State University, 1735 Neil Avenue, Columbus, OH 43206, USA
E-mail: hangarter.1@osu.edu

II. Status of National and Transnational Research Projects

1. Australia and New Zealand

New Zealand now has at least two groups working with *Arabidopsis*. One group in the Pastoral Research Institute at Palmerston North (formerly DSIR), which has a primary interest in improving white clover, is using *Arabidopsis* to identify root specific promoters for expressing resistance genes for various pests such as "grass grub". This illustrates how *Arabidopsis* research may be integrated with problems in crop plants. Another group at the School of Biological Sciences, University of Auckland, is trying to isolate genes for aluminum tolerance by screening EMS populations. A similar project is also underway at CSIRO in Canberra.

2. European Community

An EC grant has been received to set up a Concerted Action Program on plant-nematode interactions. The program has 18 participating institutes from 9 EC countries with a research focus on *Arabidopsis*. The program will not fund research directly but can be used for general meetings, frequent bilateral exchange visits, joint publications etc. In short, it is designed to stimulate nematology groups to collaborate more through the use of a common model system. As noted earlier, proposals have been called by the EC for grants to conduct large-scale sequencing of the *Arabidopsis* genome.

3. France

During the last year, *Arabidopsis* research has attracted additional French scientists and the initial effort made in the preceding year by the CNRS is beginning to bear fruits. Not only has the CNRS continued its support of *Arabidopsis* research, but now, both INRA and the Ministry of Research and Space are also funding research in this area. This Government effort helps French groups to participate actively in the world-wide project, and promising results are being produced. Various programs are now funded for the next two years and one can estimate an input of 1 million U.S. dollars per year specifically supporting *Arabidopsis* projects, not including regular grants and salaries of about 50 scientists and 50 technicians, students or postdocs. The major effect of these programs has been to encourage the various groups to work together and to integrate their research more closely with the international community, especially that from the EC.

The major scientific goal of the leading groups in France remains to study genes and their biological functions, including regulation of cell cycle, analysis of seed formation and maturation, hormone and signal transduction, response to environmental stress and gametogenesis. Two landmarks should be mentioned; the cloning by chromosome positioning of the abi3 locus by Jerome Giraudat and the isolation by complementation cloning in yeast of a gene for a potassium transporter by Herve Sentenac. For the other genes under study, most of the work is focused on characterization of promoters and gene organization within each locus.

In addition, three major programs have been launched. The first is a cDNA sequencing project mentioned earlier in this report. An important outcome of the program is that the French researchers were able to equip their laboratories with computer and automatic sequencing facilities, which should facilitate long-range mapping and sequencing. A second program, supported primarily by the Ministry of Research and Space, is designed to develop transgenic lines tagged with various insertions such as Ac, Tnt1 or various T-DNA constructs. This is part of the genetic approach to developmental biology. So far, about 3,000 independent lines have

been generated by 4 groups, but their screening is still fairly limited. Finally a third project, which is mostly concerned with stress physiology, is primarily supported by INRA.

4. Japan

Laboratories working with *Arabidopsis* have continued to increase in Japan, and now number more than 12. The research topics include mutational analyses of flower organogenesis, responses to physical and chemical stimuli, virus infection, hormonal regulation, transcriptional regulation, and transformation, as well as isolation and characterization of genes involved in heat-shock responses, desiccation, lipid biosynthesis, signaling pathways, and transcription. These studies are funded primarily by the Ministry of Education, Science, and Culture, with some grants from the Science and Technology Agency, the Ministry of Agriculture, Forestry and Fisheries, and some from private Foundations.

The training course on the standard experimental techniques on *Arabidopsis* research was held at the National Institute for Basic Biology at Okazaki on November 25 - December 1, 1991. The course included lectures, demonstration, and practice on cultivation and genetic crosses, transformation, and DNA/mRNA extraction and blotting analysis, as well as special lectures and demonstration on transformation techniques by Dr. K. Feldmann of University of Arizona and by Dr. H-G Nam of Korea, and of tissue printing technique by Dr. J. Varner of Washington University. Sixteen attendants (graduate students and young researchers) were selected from nearly 60 applicants from universities, national laboratories and private industry. Most of the attendants had no prior experience in working with *Arabidopsis*. Following the training course, the 2nd Workshop on *Arabidopsis* Studies was held on December 2 and 3, at the National Institute of Basic Biology with more than 80 participants. Dr. B. Hauge of Massachusetts General Hospital gave a talk on physical mapping at this workshop.

A book describing fundamental techniques on *Arabidopsis* cultivation, genetic analyses, and transformation was published as part of the practical laboratory technique book series written in Japanese. The training courses and meetings are planned for the coming year as well.

5. Russia

Because of a long history of using *Arabidopsis* as an experimental organism in Russia, significant collections of mutants exist. Contact has been established between Russian groups and the curators of the stock centers and a tentative agreement has been reached to transfer the Russian collection to the NASC and ABRC.

6. United Kingdom

After the success of the 1989 three-year AFRC Plant Molecular Biology Program, part of which funded 34 *Arabidopsis* project grants, a smaller program, PMB II, starts October, 1992. This will consist of nearly 40 grants, over one-third of which are expected to be on *Arabidopsis*.

7. United States and Canada

A US-Canada steering committee was elected by an electronic vote system. The steering committee consists of five members, three of whom serve on the Multinational Science Steering

Committee. The national committee will coordinate *Arabidopsis* research activities in the U.S. and Canada by facilitating open communication among researchers and serving as an advisory body to various funding agencies.

The U.S. Department of Agriculture, the Department of Energy, the National Institutes of Health, and the National Science Foundation (NSF) continue to support *Arabidopsis* research. The four agencies provided approximately $15 million during the period from October 1, 1990 to September 30, 1991 for *Arabidopsis* research. This includes new funding of $4.5 million allocated by Congress to NSF for *Arabidopsis* genome research. Most of the funds supported individual research programs covering virtually all plant biology research topics. In addition, a significant amount was spent in support of postdoctoral research fellowships, growth facilities, and the development of databases, technique/methods, and biological resources.

III. Analysis and Recommendations: New Goals for the Coming Year

The original Long-range Plan for the Multinational Coordinated *Arabidopsis* Genome Research Project (publication #: NSF 90-80) identified a series of goals for the progress of the multinational *Arabidopsis* genome project. An ongoing goal was to achieve saturation mutagenesis so as to associate a phenotype with as many genes as possible, and to develop a transposon tagging system to facilitate the cloning of genes by insertional mutagenesis. This goal continues to be realized by the explosive growth of new mutations, by the creation and distribution of large collections of tagged mutants, and by tangible progress toward identification of a useful transposon. The short-term goals were to create satisfactory YAC libraries and to link up the YACs into an ordered array. Subsequent recommendations in the first progress report included integration of the existing linkage maps, and the definition of a strategy for the collation of sequence data worldwide and for the mapping of sequenced regions to a unique physical map. Progress has been made on both fronts. New YAC libraries with larger inserts and fewer chimeric clones have been developed as technology has improved, and substantial progress has been made toward linking up the YACs. Also, the genetic maps have been consolidated and are being refined by the small-scale mapping efforts which are underway in many laboratories.

Completion of the ordered YAC maps should remain a high priority. Although many laboratories are currently utilizing YACs for chromosome walking, the consensus opinion is that walking is not a sufficiently efficient way to isolate most genes. However, if an ordered set of YACs were available, it would only be necessary to obtain high resolution mapping information for a mutation of interest in order to identify the YAC containing the gene. That would reduce the complexity of the problem to simply sorting through about 100 kb of DNA in order to identify the gene of interest by its ability to complement a mutation. In addition, as the number of cloned genes increases, the most facile way of placing cloned genes on the genetic map would be to hybridize the genes to an ordered YAC library rather than to map RFLPs associated with the gene.

At present, because of the economies of scale which can be achieved in constructing an ordered map, the work has become focused in a small number of laboratories. In view of the central importance of this project, it may be useful to consider expanding the number of laboratories involved in this project. The most efficient way of doing this is probably to coordinate the burgeoning cDNA sequencing projects with the effort to link up the YACs. That

is, in order to identify the function of most randomly sequenced cDNAs, it will be necessary to obtain additional information about the clones such as genetic map location. The only feasible strategy for accomplishing this is to hybridize the cDNA clones to YAC filters, or vice versa. Whenever the cDNA clone hybridizes to two YAC clones in a region of overlap, information on linkage of the YACs will result. Thus, by hybridizing several thousand cDNA clones to the YAC libraries, it will be possible to link up the YACs. Therefore, we recommend that support be allocated to large-scale mapping of partially sequenced cDNA clones.

A long term goal of the genome project was the complete sequencing of all cDNAs and the mapping of these cDNAs on the genetic map. This aspect of the project has been initiated with the establishment of one US laboratory dedicated to cDNA sequencing, and at least seven European laboratories with this goal. In view of recent improvements in automated sequencing instruments, it is apparent that with a modest investment, it is possible to obtain partial sequence information on a majority of the estimated 20,000 mRNAs in *Arabidopsis* within the next several years. In view of the fact that it has become possible to identify an increasing number of deduced amino acid sequences by sequence identity or homology to polypeptides of known function, this project promises to be very productive. As noted above, mapping of partially sequenced cDNAs should be an integral part of this aspect of the project. We recommend that the cDNA sequencing and mapping be assigned support with the caveat that suitable mechanisms be put in place to ensure that the entire community has rapid and unrestricted access to the results of all large-scale sequencing efforts. As noted below, additional cooperation must be implemented to avoid wasteful duplication of effort in this enterprise.

Because of the importance of an accurate genetic map to all map based cloning efforts, it is recommended that support be allocated to the establishment of a genetic map committee which would be responsible for collecting all sequence information and integrating the data into a consensus map which would be distributed via the databases and newsletters.

A result of the US-EC Sequence Database Workshop in January 1992 was the recognition that there are a number of important issues pertaining to the organization of international *Arabidopsis* nucleotide sequence databases that should be resolved in the immediate future by subsequent meetings. In particular, a central database should be designated and the design of data descriptors and nomenclature resolved before a large stream of data has already been acquired. Issues pertaining to release of information from individual laboratories must be resolved and some attempt to utilize the same biological materials as sources of DNA should be agreed upon.

In the original goals of the project, a high priority was assigned to the creation of biological resource centers. Two stock Centers and two DNA repositories are now operating and the preliminary evidence indicates that they will serve an important role in ensuring rapid and continuing access to the biological materials and information as they are developed. The development of two databases is also an important step forward in providing rapid access to the explosion of information. We strongly endorse continuing support for these resources. In the area of communications, the *Arabidopsis* newsgroup has become an important mechanism for discussion of issues and the sharing of technical information. The success of this important medium must be attributed in large part to the responsive and efficient contribution of Dave Kristofferson, the Genbank manager of the BIOSCI network. It is to be hoped that support for the BIOSCI network will remain strong and unabated. The one remaining aspect of communication which must be addressed in the near future is the creation of a newsletter. The newsletter is expected to differ from the electronic newsgroup by providing a readily accessible

record of selected items from the electronic newsgroup, and by the inclusion of concise notes about scientific observations which are of relevance primarily to the *Arabidopsis* community. We anticipate that a newsletter will appear in the forthcoming year and encourage anyone who would be interested in participating as an editor to contact a member of the Multinational Science Steering Committee.

In addition to the progress described in this report, *Arabidopsis* research has undergone a major transition during the past several years. In effect, the organism has become the most widely utilized experimental organism in experimental plant biology. Thus, to present a full account of *Arabidopsis* research has become very similar to summarizing progress in plant biology. The implication in the present context is that it suggests that in order to avoid losing sight of the goals of the genome project, it may be appropriate to begin focusing the project more narrowly on the goals which are directly related to the *Arabidopsis* genome. In particular, we believe it is time to begin allocating increased support for large-scale sequencing of both cDNA and genomic clones.

In the area of human resource development, the ongoing goals which we continue to endorse, were the support of multinational postdoctoral fellowships and short term exchanges and short term courses.

An important aspect of research on *Arabidopsis* has been the spirit of cooperation and openness which has characterized the field. The *Arabidopsis* genome project will generate many unique research resources (nucleotide sequences, mapping data, clones, strains, databases and software). Some mechanisms have now been established to facilitate free and unrestricted access to these resources by all members of the international committee. It is to be hoped that all participants in *Arabidopsis* research, regardless of their affiliation, will continue to make their research results and accomplishments readily available. One mechanism which we strongly endorse is the deposition of samples of new mutants, libraries and cloned genes in the resource centers.

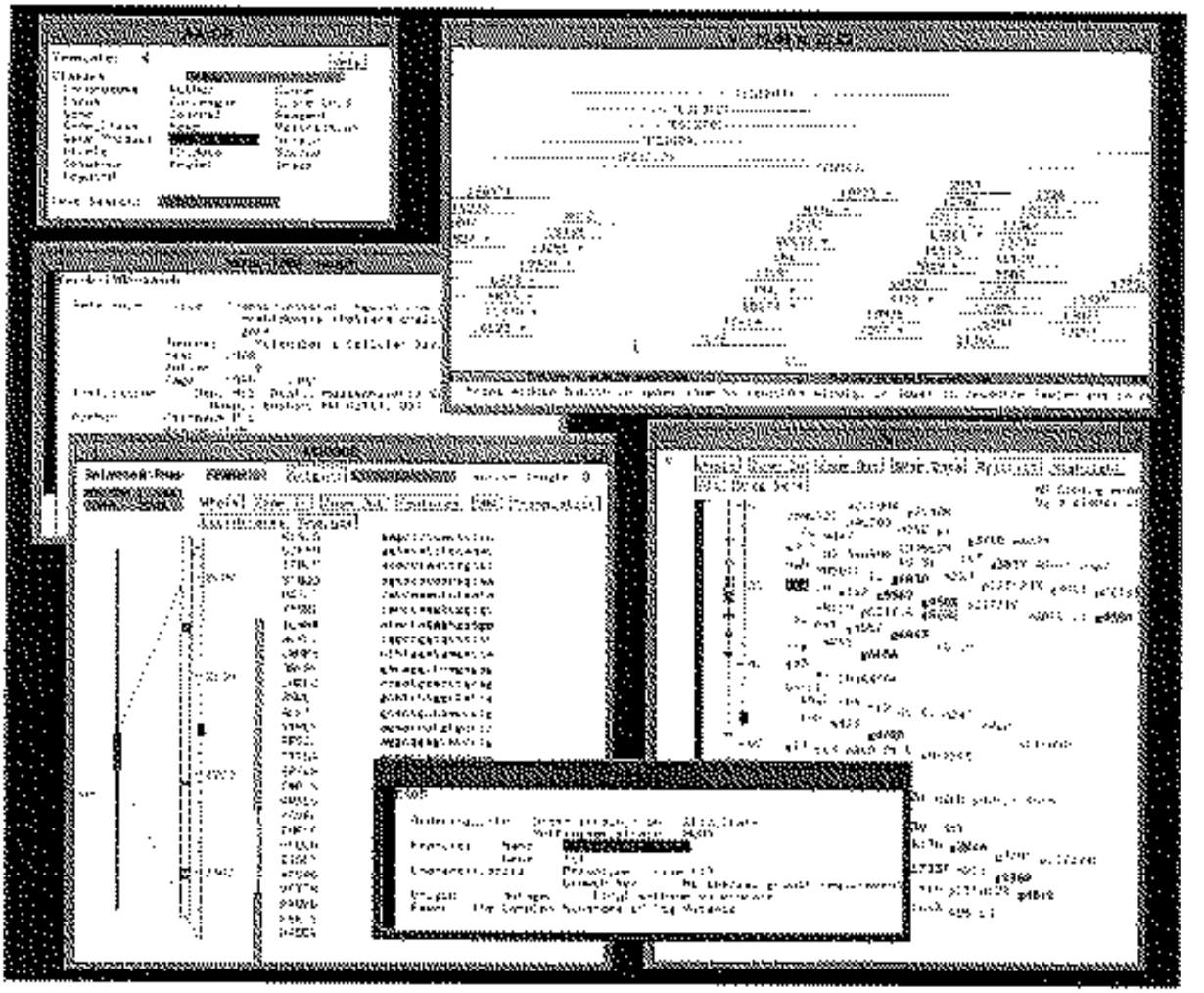
Progress in *Arabidopsis* research has occurred, in part, because *Arabidopsis* is integrated into general genome and plant biology research activities. The *Arabidopsis* research community has been quick to take advantage of any new developments in biological sciences, and at the same time, information and knowledge gained through studies of *Arabidopsis* have been utilized by the general biological research community. Major progress on many previously intractable problems has been made using *Arabidopsis* as a model system and we anticipate that we will soon begin to see the application of these advances to directed improvement of plants of economic significance. It is, therefore, of great importance that mechanisms for interrelating the information gained in *Arabidopsis* research and research on other plants be fostered. One promising mechanism for facilitating this interplay will be the development of common standards in the databases which are being developed for many crop species such as maize, wheat, soybean and tomato. Another facilitating mechanism is to ensure that committees which review grant proposals and related matters concerning research in crop species include members of the *Arabidopsis* community who can provide information about the current developments in *Arabidopsis* research. For similar reasons, membership on committees which deal primarily with *Arabidopsis* research should continue to have representation by colleagues with primary research interests which extend beyond *Arabidopsis*.

Finally, we recommend continued and expanded commitment to all of the goals set forth for the Multinational *Arabidopsis thaliana* Genome Research Project. Funding agencies around the world should continue to recognize the value of the truly international nature of the

Arabidopsis genome project, and allocate sufficient resources to help achieve the important international goals.

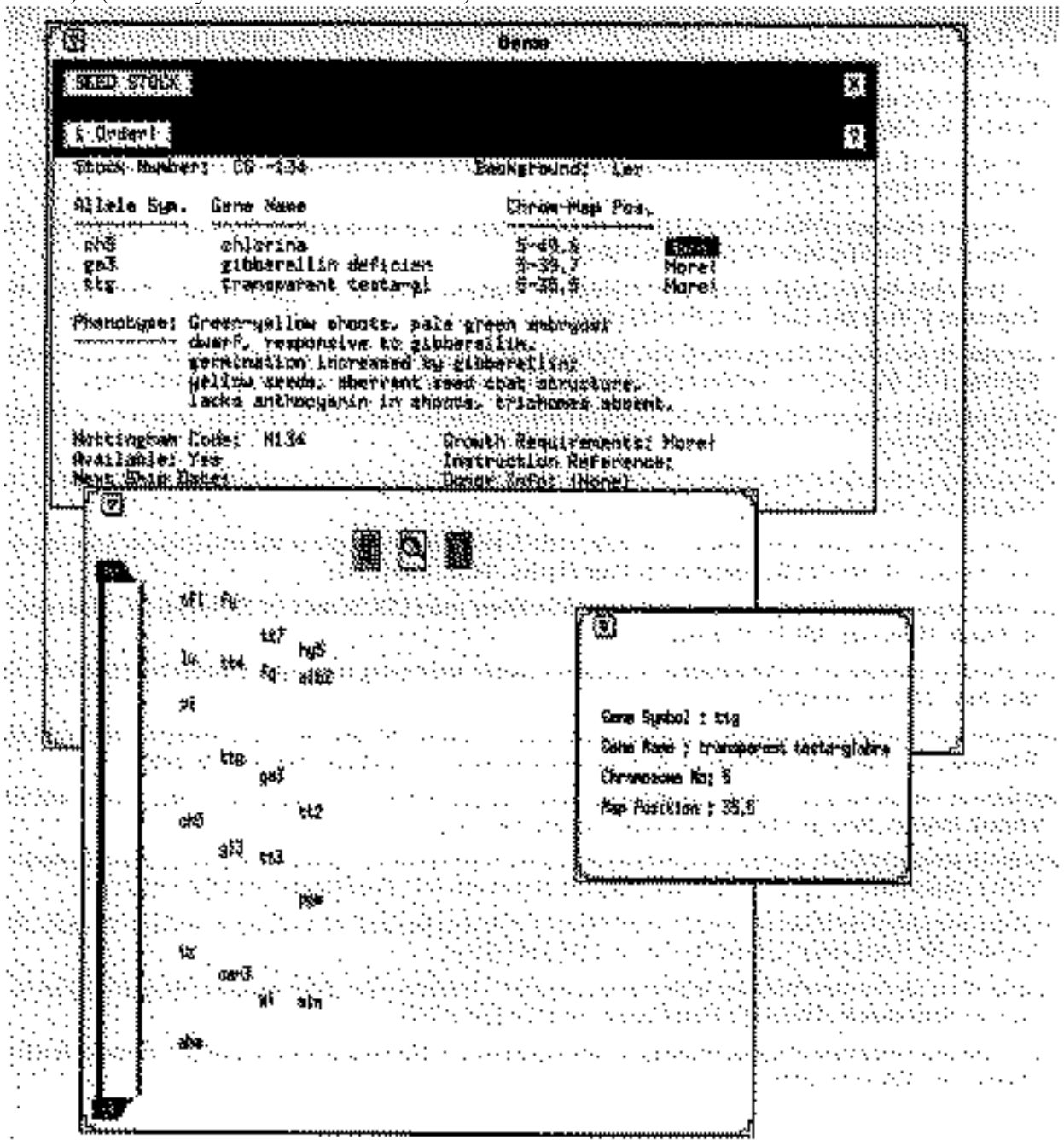
Appendix I: AAtDB Example Display

This figure illustrates examples of the windows used by the ACeDB software developed by Drs. Richard Durbin and Jean Thierry-Mieg to display the many types of research information contained within the *Arabidopsis thaliana* database, AAtDB. Clockwise from upper left: AAtDB main window listing the different topics included in the database, YAC and cosmid physical map correlated to the genetic map, unified *Arabidopsis* genetic map of RFLP and classical markers, strain descriptions and ordering information for both the ABRC at Ohio State and the Nottingham Stock Center in the U.K., protein sequences with features, and finally, extensive bibliographic citations. Version 1.1 of AAtDB was released to the public in early July, 1992, after three months of beta testing at *Arabidopsis* laboratories around the world. (Courtesy of Dr. Michael Cherry.)



Appendix II: AIMS Sample Screen

AIMS (*Arabidopsis* Information Management System) contains detailed information on many aspects of *Arabidopsis*. Included are data on DNA and seed stocks (see table at the top of sample screen) and the facility for direct order placement. The graphic display of genetic maps, etc. also is possible (see map of available chromosome 5 mutants at the bottom of sample screen). (Courtesy of Dr. Sakti Pramanik.)



Appendix III:

Partial Listing of Recent *Arabidopsis* Publications

The following represents a partial listing of Arabidopsis research papers published from January 1991 to August 1992. This list was downloaded from "Reference Update" (Research Information Systems, Inc., Camino Corporte Center, 2355 Camino Vida Roble, Carlsbad, CA) and updated by Chris Somerville.

cDNA clones encoding *Arabidopsis thaliana* and Zea mays mitochondrial chaperonin HSP60 and gene expression during seed germination and heat shock, Prasad,T.K., Stewart,C.R. Plant Mol. Biol., 18:873-885 (1992)

Characterization of rps17, rpl9 and rpl15: Three nucleus-encoded plastid ribosomal protein genes, Thompson,M.D., Jacks,C.M. Lenvik,T.R., Gantt,J.S. Plant Mol. Biol., 18:931- 944 (1992)

Identification of an *Arabidopsis* DNA-binding protein with homology to nucleolin, Didier,D.K., Klee,H.J. Plant Mol. Biol., 18:977-979 (1992)

An *Arabidopsis thaliana* cDNA clone encoding a 17.6 kDa class II heat shock protein, Bartling,D., Buelter,H., Liebeton,K. Weiler,E.W. Plant Mol. Biol., 18:1007-1008 (1992)

A new homeobox-leucine zipper gene from *Arabidopsis thaliana*, Mattsson,J., Soederman,E., Svenson,M., Borkird,C., Engstroem, P. Plant Mol. Biol., 18:1019-1022 (1992)

Ion channels in *Arabidopsis* plasma membrane. Transport characteristics and involvement in light-induced voltage changes, Spalding, E.P., Slayman,C.L., Goldsmith,M.H.M., Gradmann,D., Bertl,A. Plant Physiol., 99:96-102 (1992)

A role for membrane lipid polyunsaturation in chloroplast biogenesis at low temperature, Hugly,S., Somerville, C.R. Plant Physiol. 99:197-202 (1992)

Isolation and characterization of a mutant of *Arabidopsis thaliana* resistant to Alpha-methyltryptophan, Kreps,J.A., Town,C.D. Plant Physiol., 99:269-275 (1992)

An *Arabidopsis thaliana* gene with sequence similarity to the S-locus receptor kinase of Brassica oleracea. Sequence and expression, Tobias,C.M., Howlett,B., Nasrallah,J.B. Plant Physiol., 99:284-290 (1992)

Exon-intron organization of the *Arabidopsis thaliana* protein kinase genes CDC2a and CDC2b, Imajuku,Y., Hirayama,T., Endoh, H., Oka,A. FEBS Lett., 304:73-77 (1992)

Genes encoding a histone H3.3-like variant in *Arabidopsis* contain intervening sequences, Chaubet,N., Clement,B., Gigot,C. J. Mol. Biol., 225:569-574 (1992)

Identification of two tungstate-sensitive molybdenum cofactor mutants, chl2 and chl7, of *Arabidopsis thaliana*, LaBrie,S. T., Wilkinson,J.Q., Tsay,Y.-F., Feldmann,K.A., Crawford,N.M. Molec. Gen. Genet., 233:169-176 (1992)

Expression of variant nuclear *Arabidopsis* tRNASer genes and pre-tRNA maturation differ in HeLa, yeast and wheat germ extracts, Beier,D., Beier,H. Molec. Gen. Genet., 233:201-208 (1992)

Phytoalexin accumulation in *Arabidopsis thaliana* during the hypersensitive reaction to Pseudomonas syringae pv syringae, Tsuji,J., Jackson,E.P., Gage,D.A., Hammerschmidt,R., Somerville, S.C. Plant Physiol., 98:1304-1309 (1992)

Role of abscisic acid in the induction of desiccation tolerance in developing seeds of *Arabidopsis thaliana*, Meurs,C., Basra, A.S., Karssen,C.M., Van Loon,L.C. Plant Physiol., 98:1484-1493 (1992)

H-protein of the glycine decarboxylase multienzyme complex. Complementary DNA encoding the protein from *Arabidopsis thaliana*, Srinivasan,R., Oliver,D.J. Plant Physiol., 98:1518-1519 (1992)

Isolation and characterization of a gene encoding a carboxypeptidase Y-like protein from *Arabidopsis thaliana*, Bradley,D. Plant Physiol., 98:1526-1529 (1992)

Complementary DNA sequence of a low temperature-induced Brassica napus gene with homology to the *Arabidopsis thaliana* kin1 gene, Orr,W., Iu,B., White,T.C., Robert,L.S., Singh,J. Plant Physiol., 98:1532-1534 (1992)

LEAFY controls floral meristem identity in *Arabidopsis*, Weigel, D., Alvarez,J., Smyth,D.R., Yanofsky,M.F., Meyerowitz,E.M.: Cell, 69:843-859 (1992)

A genetic model for light-regulated seedling development in *Arabidopsis*, Chory,J. Development, 115:337-354 (1992)

Germinal and somatic activity of the maize element Activator (Ac) in arabidopsis, Keller,J., Lim,E., James,D.W.,Jr., Dooner, H.K. Genetics, 131:449-459 (1992)

Strategies for mutagenesis and gene cloning using transposon tagging and T-DNA insertional mutagenesis, Walbot,V. Ann. Rev. Plant. Physiol. Plant Mol. Biol., 43: 49-82 (1992)

Fusion events during floral morphogenesis, Verbeke,J.A. Ann. Rev. Plant. Physiol. Plant Mol. Biol., 43:583-598 (1992)

Mapping and sequencing of an actively transcribed Euglena gracilis chloroplast gene (ccsA) homologous to the *Arabidopsis thaliana* nuclear gene cs(ch-42), Orsat,B., Monfort,A., Chatellard,P. Stutz,E. FEBS Lett., 303:181-184 (1992)

A 126 bp fragment of a plant histone gene promoter confers preferential expression in meristems of transgenic *Arabidopsis*, Atanassova,R., Chaubet,N., Gigot,C. Plant J., 2:291- 300 (1992)

Complementation of Saccharomyces cerevisiae auxotrophic mutants by *Arabidopsis thaliana* cDNAs, Minet,M., Dufour,M.-E., Lacroute, F. Plant J., 2:417-422 (1992)

Light-induced phosphorylation of a membrane protein plays an early role in signal transduction for phototropism in *Arabidopsis thaliana*, Reymond,P., Short,T.W., Briggs,W.R., Poff,K.L.: Proc. Natl. Acad. Sci. USA

Genes that regulate plant development, Aeschbacher,R.A., Benfey, P.N. Plant Sci., 83:115-126 (1992)

Functional expression of a plant plasma membrane transporter in Xenopus oocytes, Boorer,K.J., Forde,B.G., Leigh,R.A., Miller, A.J. FEBS Lett., 302:166-168 (1992)

Identification of a single-copy gene encoding a Type I chlorophyll a/b-binding polypeptide of photosystem I in *Arabidopsis thaliana*, Jensen,P.E., Kristensen,M., Hoff,T., Lehmbeck,J., Stummann, B.M., Henningsen,K.W.

Physiol. Plant., 84:561-567 (1992)

Analysis of multiple photoreceptor pigments for phototropism in a mutant of *Arabidopsis thaliana*, Konjevic,R., Khurana, J.P., Poff,K.L. Photochem. Photobiol., 55:789- 792 (1992)

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