

# Arabidopsis Blooms

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## MEETING REPORT

From October 12 to 15, 1989, approximately 400 scientists gathered in Bloomington, Indiana, to report recent progress made in harnessing *Arabidopsis* to the problems of plant biology. Representatives from 82 laboratories displayed 95 posters and gave 42 oral presentations. The subject matter was diverse. However, because there are specific technical reasons for choosing *Arabidopsis* as the experimental organism, there were several prevalent themes that are selectively illustrated by the following overview.

The essential problem with the genetic approach to problems in plant growth and development traditionally has been the inability to make the connection between phenotype and genotype. Although geneticists have collected many mutations affecting a wide range of characters, it has not been possible to identify the primary lesion except in those few cases where the mutation was specifically selected in a well-characterized biochemical pathway or was due to insertion of a transposable element. This is no longer the case for those utilizing *Arabidopsis* because of an important step made toward realizing the full potential of the organism as an experimental system. Tony Bleecker and collaborators in the laboratory of Elliot Meyerowitz (Caltech, Pasadena, CA) presented preliminary evidence indicating that they have identified a cosmid carrying the dominant *etr* mutation from an ethylene-insensitive mutant of *Arabidopsis* (Bleecker et al., 1988). Seedlings descended from a transgenic plant that had been transformed with DNA from the *etr* mutant gained the ethylene-insensitive phenotype of the mutant. Thus, if substantiated by further analysis, we may soon expect to have in hand the first gene product that demonstrably participates in the response of a higher plant to a growth regulator.

Aside from the importance of the result for understanding growth regulation, the result is a landmark because it signals the arrival of a new phase in plant biology. The *etr* gene was isolated by walking along the chromosome from flanking restriction fragment length polymorphisms (RFLPs). Work from other laboratories indicates that this is just the tip of the iceberg. During the past several years, Brian Hauge and collaborators in the laboratory of Howard Goodman (Massachusetts General Hospital, Boston) have been involved in the gargantuan effort of linking up the entire genome of *Arabidopsis* by using combinatorial analysis to identify overlapping cosmids (contigs) on the basis

of common restriction fragments (Coulson et al., 1986). Approximately 18,000 cosmid preparations have been analyzed, but, as in the case of similar experiments with the nematode genome, the number of contigs cannot be resolved below about 800. Nevertheless, in conjunction with the RFLP maps constructed (and distributed) by the Meyerowitz and Goodman laboratories (Chang et al., 1988; Nam et al., 1989), the availability of the contigs should greatly facilitate chromosome walking. As a demonstration of the potential, Jerome Giraudat and Hauge presented RFLP mapping evidence indicating that cosmids covering the *abi3* (abscisic acid-insensitive) and *ga2* (gibberellin biosynthesis) loci have been identified.

Although still tedious, chromosome walking has recently become much easier because of the development of methods for cloning large fragments of DNA as artificial chromosomes in yeast (YACs) (Burke, Carle, and Olson, 1987). Several groups have constructed YAC libraries carrying relatively large fragments of *Arabidopsis* DNA. Erwin Grill (Michigan State University, Lansing) described a library, in use in a number of laboratories, in which a collection of 2400 YACs with an average insert size of 150 kb represents approximately three *Arabidopsis* genomes. By sampling for the presence of YACs corresponding to randomly chosen RFLPs, it appears that most of the genome is represented in the library. Grill noted that it should be possible to obtain YACs covering 40% of the genome simply by identifying those YACs that hybridize to the probes used to construct the existing RFLP maps. Thus, it seems likely that it will not be long until a collection of YACs spanning the entire genome is available.

The concept of linking up the genome with cloned DNA fragments, which would have been considered impossible a few years ago and now seems relatively straightforward, serves as a useful reminder that technical advances are frequently surprising. This is the underlying assumption (perhaps hope is a better word) of the groups who have initiated genome projects for their favorite organisms. Because plants were omitted from the original list of acceptable model organisms for the National Institutes of Health (NIH)-sponsored human genome project, the National Science Foundation (NSF) has taken up the banner and sponsored several small workshops concerning the possibility of an *Arabidopsis* genome project. (NIH currently supports at least one *Arabidopsis* genome project.) DeLill

Nassar (NSF) presented a brief overview of the results of the first three meetings. Basically, it appears that NSF will try to obtain funds to support one or more resource centers to distribute clones, RFLP markers, YACs, and seeds; compile a database; and undertake a broad approach to characterizing the *Arabidopsis* genome. At this point the goal is to provide support for the project over a period of 10 years and to have the project culminate in the completion of the sequencing of the genome.

Isolating genes by chromosome walking is somewhat like playing the lottery by buying all the tickets: it is a useful approach if the value of winning exceeds the cost. However, most geneticists have traditionally accepted the risks associated with less controlled methods. In this respect there has been very substantial progress in isolating interesting genes using a method described several years ago by Ken Feldmann and David Marks, in which treatment of germinating seeds of *Arabidopsis* with *Agrobacterium tumefaciens* led to transformation of some of the resulting progeny (see Feldmann et al., 1989). Approximately 20% of the random T-DNA inserts generate phenotypes. By screening 50,000,000 "T2 progeny," Feldmann and colleagues at Dupont (Wilmington, DE) have obtained about 1500 transformants, of which about 300 have visible phenotypes that are thought to be due to T-DNA inserts. The work is a veritable bonanza for those working on many aspects of development. For instance, at least 15 of the mutants apparently have phenotypes similar to the embryo lethal mutants studied in detail by David Meinke and collaborators (University of Oklahoma, Stillwater). Feldmann and colleagues have generously provided many of the mutants to other laboratories, and the first results of these collaborations are very exciting. Among the first genes to be cloned by this method was a mutant allele of the agamous (*ag*) locus, which has the homeotic phenotype of a flower within a flower. Martin Yanofsky and collaborators in Meyerowitz's lab used the DNA surrounding T-DNA in an *ag* mutant generated by Feldmann to clone the gene and have complemented the mutation with the corresponding segment of wild-type DNA. The gene encodes a 1.2-kb RNA that appears to be preferentially expressed in flowers. The reading frame contained a 73-amino acid subsequence that, allowing for conservative amino acid substitutions, has about 85% sequence identity with at least three eukaryotic transcription factors (SRF, human serum response factor involved in regulation of *c-fos*; MCM1, a yeast transcription factor involved in mating; and the yeast transcription factor ARG80).

Another gene that has been "Ti-tagged" and cloned was described by Patricia Herman and David Marks (University of Nebraska, Lincoln) (see Marks and Feldmann, 1989; Herman and Marks, 1989). The *gl1* locus controls the presence of trichomes on the leaf surface and other tissues. The locus is interesting because it presumably specifies the terminal differentiation of a unique cell type from among a field of similar epidermal cells. An 8.3-kb DNA

fragment from the wild type complemented a *gl1* mutant. The most commonly available mutant alleles of *gl1* lack trichomes on both leaves and stems. Interestingly, the mutant with the T-DNA insert was lacking trichomes only on the stem and had normal trichomes on the leaves, suggesting that the insert disrupted a region involved in tissue-specific expression.

Because the seed transformation method has been somewhat fickle, other laboratories have also been exploring T-DNA mutagenesis by exploiting recent improvements in *Arabidopsis* transformation of tissue explants (Valvekens, Van Montagu, and Van Lijsebettens, 1988). Csaba Koncz (Max-Planck-Institut, Köln, Federal Republic of Germany) described the production of 12,000 transgenic shoots of *Arabidopsis*. The T-DNA used for these experiments utilized a promoterless *aph(3')*II reporter gene linked to the right end of the T-DNA. Transformants were first selected for hygromycin resistance and 450 fertile plants regenerated. Surprisingly, the frequency with which promoter fusions were found in *Arabidopsis* and *Nicotiana* was very similar, suggesting that T-DNA preferentially inserted into transcriptionally active genes. Another surprising result was the low frequency of mutations found in the progeny of the 450 transgenic plants. By contrast with the 19% reported by Feldmann et al. (1989), Koncz observed mutations in only about 1% of the transformants, indicating a fundamental and mysterious difference between the two methods. One of the T-DNA inserts was found to have created a new allele of a chlorina mutation at the *ch-42* locus. Although not presented at this meeting, a similar approach has been used by Jack Okamoto and colleagues in the laboratory of Van Montagu (Rijksuniversiteit, Gent, Belgium) to generate approximately 1000 transformants. At the recent EMBO symposium in Heidelberg during September 1989, Okamoto presented evidence indicating that a T-DNA insert had cosegregated with a new allele of the *apetella-2* (*ap2*) locus. This locus, which was the subject of at least four presentations, is particularly interesting because various alleles exhibit unique patterns of homeotic transformation of the various perianth organs (Kunst et al., 1989).

Substantial effort has been invested by several laboratories in developing Ac as a gene-tagging system in *Arabidopsis*. Although many groups have observed the presence of somatic excision of Ac derivatives in transgenic plants, there has not been evidence for germinal excision. Bob Masterson (Max-Planck-Institut, Köln) and Renate Schmidt (Institut für Genbiologische Forschung, West Berlin) presented evidence that, in certain Ac derivatives, germinal excision events occur at a frequency of about 0.2% to 0.5%. Excision was accompanied by reinsertion of the element, suggesting that the system can be used for insertion mutagenesis. However, it appears that a substantial amount of work needs to be done to refine the system. Results presented by Schmidt and a poster by Innes, Baker, and Staskawicz (University of California,

Berkeley, and USDA Plant Gene Expression Center, Albany, NY) provided evidence that Ds will excise when activated by the Ac transposase in *trans*. Promising work was also presented by Caroline Dean (John Innes, Norwich, United Kingdom), indicating that removal of most of the nontranslated leader sequence of an Ac element led to a very high frequency of somatic excision as evidenced by the profusion of streptomycin-resistant (i.e., green) sectors on the leaves of transgenic plants carrying the modified element in the leader sequence of a gene for streptomycin resistance. Thus, although a mutant has yet to be produced, the approach remains a promising alternative to Ti-tagging and chromosome walking. Several laboratories are also exploring the use of Tam3. At the recent EMBO meeting, Jack Okamoto presented preliminary evidence that Tam3 excises in *Arabidopsis*.

It has been apparent for some time that discoveries made using *Arabidopsis* and other plants will increasingly have an impact on scientists working with eukaryotes from other orders. A notable example of this trend was a presentation by Witold Filipowicz (Friedrich Miescher Institute, Basel, Switzerland), concerning the promoters of the U-snrRNA gene families of *Arabidopsis*. The U2 and U5 RNA genes are transcribed by RNA polymerase II (pol II), whereas U6 is transcribed by pol III. Filipowicz reported the surprising discovery that it was possible to convert the pol III promoter into a pol II promoter, and vice versa, simply by changing the spacing between the elements by addition or deletion of 10 bp of DNA! However, for the moment, there remains much to be learned about higher plants by adapting specific information gained in other systems. In this context, Gerry Fink (MIT, Cambridge, MA) noted that yeast genes are frequently useful probes for the isolation of the corresponding *Arabidopsis* genes. The list of genes cloned in this way now includes acetolactate synthase, anthranilate synthase, the  $\beta$ -subunit of tryptophan synthase, a putative endoplasmic reticulum  $\text{Ca}^{2+}$  ATPase, myoinositol 1-phosphate synthase, HMG CoA reductase, and ribosomal protein L3. In addition, the observation that a cDNA clone for *Arabidopsis* HMG CoA reductase will complement a yeast mutant indicates that it should be possible to clone some genes by selecting for plasmids that complement the yeast mutants.

Most of the recent enthusiasm for *Arabidopsis* has been founded on the technical advantages of the small genome size. However, what will sustain the use of the organism is the isolation of mutants that define interesting genes. In this respect there was ample evidence that the field is developing apace. Indeed, much of the meeting was devoted to the description of new mutants. A personal favorite was a mutant isolated by Kiyotaka Okada and colleagues (National Institute of Basic Biology, Okasaki, Japan) as part of a project concerned with the mechanism by which roots avoid impediments. When the tip encounters an obstacle, the root changes the direction of elongation by rotating the tip alternately right and left. By

growing seedlings on the surface of Petri dishes containing a high agar concentration and placed at a 45° angle, the periodic switching pattern of growth was induced and mutants with an altered response were identified. In the most extreme case, a mutant was identified that was unable to reverse the direction of the avoidance response. As a result the roots form a tight right-hand coil on the inclined agar surface.

Several other groups have also exploited the fact that *Arabidopsis* roots grown at relatively high density on the surface of vertically oriented Petri dishes can be readily observed. John Schiefelbein (Michigan State University, Lansing) described a collection of about 80 mutants with altered root morphology culled by visually examining the roots of about 12,000 M2 plants. Some of the mutants lack root hairs or have bulbous, branched, or distorted hairs. Other mutants have dwarf roots or other morphological abnormalities of the roots, but normal shoots. Similar mutants were also reported by Richard E. Williamson and colleagues (Australian National University, Canberra), who used immunofluorescent staining of the cortical microtubules to show that the mutants with bulging root hairs are associated with a disorganization of the microtubule-microfibril mechanisms that normally regulate cell shape. Some of the mutants were similar in phenotype to the root-hairless *axr2* mutants described by Allison Wilson (Indiana University, Bloomington), which were recovered by selection for resistance to indole acetic acid. Thus, root hairs may prove to be a particularly attractive system for making a mechanistic link between auxin and some of the mechanisms that regulate cell shape.

Mutations affecting the action of the other growth regulators were a major theme. Joe Ecker (University of Pennsylvania, Philadelphia) reported the identification of a collection of new mutants with altered response to ethylene. One interesting mutant (*eto1*) mimics the response of wild-type plants to exogenous ethylene (i.e., the apical hook becomes exaggerated while elongation of the hypocotyl and root is inhibited). This mutant produced at least 60-fold more ethylene than wild type. Several mutants that were insensitive to ethylene were also isolated and mapped. One mutant uncoupled the response of the hook from root and hypocotyl elongation. A novel class of mutants affecting the mechanisms involved in the light-dependent development of chloroplasts was described by Joanne Chory (Salk Institute, La Jolla, CA). One class (*det*) developed true leaves in the absence of light. The mutants displayed many of the characteristics that are light-dependent in the wild type, including leaf and chloroplast development, anthocyanin accumulation, and accumulation of mRNAs for several light-regulated nuclear and chloroplast genes (e.g., *rbcS*, *rbcL*, *psbA*, *psaA*). Several additional mutations that result in the long-hypocotyl (*hy*) phenotype were also described. There are now seven complementation groups, three of which show deficiencies in photoreversible phytochrome activity and are thought

to be signal perception mutants (Chory et al., 1989). The isolation of the corresponding genes should provide a useful new approach to understanding the mode of action of phytochrome.

Unfortunately, it is not possible to describe all of the kinds of mutants being worked on. The list of phenotypes and characters for which mutants are available included homeotic mutants and other classes of mutations affecting morphological development, late-flowering mutants (more than 40 mutations at 11 loci), mutants with altered gravitropism and phototropism, mutants altered in response to abscisic acid or gibberellin, embryo lethal mutants, photosynthetic-deficient mutants, herbicide-resistant mutants, male-sterile mutants, mutations in purine metabolism, catalase-deficient mutants, amino acid and biotin auxotrophs, variegated mutants, salt-tolerant mutants, cesium-resistant mutants, analog-resistant mutants, mutants deficient in glucosinolate biosynthesis, starchless mutants, and at least a dozen loci involved in regulating lipid composition. Perhaps the most impressive example of mutant isolation was the report by Ted Underhill, George Haughn, and collaborators (National Research Council, Saskatoon, Saskatchewan, Canada) of mutants deficient in glucosinolate biosynthesis by screening an M2 population by HPLC analysis of tissue extracts. This is, presumably, the biochemists' equivalent of chromosome walking.

In general, the other model organisms that are commonly used for the study of development at the molecular genetic level are poorly characterized at the biochemical and physiological levels. *Arabidopsis* will be an exception to this rule, as there was ample evidence that the biochemistry and physiology are being studied vigorously. In reference to this point, one wag noted that *Arabidopsis* was blessed with the equivalents of both psychiatrists (i.e., developmental biologists) and proctologists (i.e., biochemists). A good example of the potential overlap between these disciplines was presented by Mike Sussman (University of Wisconsin, Madison), who described the role of the plasma membrane  $H^+$  ATPase in regulating the membrane channels and carriers that presumably mediate many cellular aspects of development (Sussman and Harper, 1989). In this respect, the existence of at least three closely related genes for the  $H^+$  ATPase raises the interesting possibility of functional polymorphism among this class of proteins.

Ion channels were also a theme in a talk by Janet Braam (Stanford, Palo Alto, CA) on rain-, wind-, and touch-induced expression of calmodulin genes. Within 10 to 30 minutes after stimulation, the mRNA levels of at least five genes increase by as much as 100-fold and then decrease to resting-state level within several hours. The response does not appear to be the long-awaited explanation of why plants reportedly favor certain kinds of music but might be important in physiological adaptation to certain forms of environmental stress. It was suggested that the phenom-

enon could be related to activation of stretch-activated calcium channels.

Adaptation to thermal or cold stress was the subject of several presentations. If given a period of adaptation to low non-freezing temperature, *Arabidopsis* will reportedly survive to at least  $-14^{\circ}\text{C}$ . Mike Thomashow (Michigan State University, Lansing) described the isolation of four genes encoding a family of proteins that are strongly induced following transfer of *Arabidopsis* to low temperature. Run-on transcription assays indicated that cold-induced mRNA accumulation for three of the genes is regulated post-transcriptionally. A key to their function seems to be that none of the gene products is precipitated from solution by boiling. The deduced sequence of at least one of the genes showed strong homology to a family of proteins that accumulate late during cotton embryogenesis (Baker, Steele, and Dure, 1988), suggesting a role in desiccation.

Under the assumption that this year's interesting poster is next year's breakthrough, it seems likely that *Arabidopsis* may be a useful system for gaining some insights into the molecular basis of plant-pathogen interactions. Although there has been tremendous progress in isolating genes that are involved in pathogenesis from plant pathogens, there has not been comparable progress on the plant side. Several groups presented preliminary results concerning the development of plant pathogen systems in *Arabidopsis*. Keith Davis, Fred Ausubel, and colleagues (Ohio State University and Massachusetts General Hospital) reported that several genes (e.g., phenylalanine ammonia lyase, caffeic acid *o*-methyltransferase, and  $\beta$ -1,3-glucanase) are induced in leaves infiltrated with bacterial strains of *Pseudomonas syringae* pv *maculicola* that are either pathogenic or induce a hypersensitive reaction. Tapio Palva (Swedish University of Agricultural Science, Uppsala) reported similarly that treatment of *Arabidopsis* with exoenzymes from *Erwinia carotovora* induces several pathogenesis-related proteins and that this treatment induces resistance to subsequent infection. Jun Tsuji and Shauna Somerville (Michigan State University, Lansing) reported the identification of a single dominant allelic difference between two races of *Arabidopsis* that confers resistance to chlorosis induced by *Xanthomonas campestris* pv *campestris*. Brian Staskawicz and colleagues (University of California, Berkeley) indicated that they had also identified genetic differences for resistance to *P. syringae*. Somewhat surprisingly, only one poster by José Martínez (CIT-INIA, Madrid, Spain) described studies of viral pathogenesis of *Arabidopsis*. Martínez and collaborators have identified an induced mutation that shows altered symptoms in response to infection with turnip yellow mosaic virus. They also observed that the ethylene-resistant *etr* mutant showed different symptoms to TYMV infection.

By the time the meeting ended there was a general feeling that, in the 2 years since the last *Arabidopsis*

meeting, there has been an important transition in the kind of research being done with *Arabidopsis*. It is no longer simply convenient to work with *Arabidopsis*, it is now possible to undertake experimental approaches to problems that are not feasible with any other organism. Anything that can be marked with a mutation is now accessible to experimental analysis at the molecular level. I eagerly anticipate the next meeting of the group in Vienna, June 2–5, 1990.

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