

IN THIS ISSUE

Giving Rice the Time of Day: Molecular Identification of a Major Photoperiod Sensitivity Quantitative Trait Locus

Daylength controls flowering in many plant species. Early in the twentieth century, Garner and Allard (1920) first described the phenomenon of plants that flower only when daylength is longer or shorter than a particular threshold, named long-day and short-day plants, respectively. Rice is a short-day plant; flowering (termed "heading" in rice and other cereals) is promoted by short daylength. However, there is a large degree of genetic variability in this trait among rice cultivars, from photoperiod insensitivity to strong photoperiod sensitivity. The manipulation of photoperiod sensitivity is an important breeding objective for rice grown in many tropical and temperate regions to optimize heading date and maturity for local environments (Poonyarit et al., 1989; Li et al., 1995).

Like many other important traits in plant breeding, heading date is a complex trait that shows continuous phenotypic variation among progeny and is controlled by multiple genes known as quantitative trait loci (QTLs). QTLs typically are difficult to identify because of the lack of discrete phenotypic segregation and because the phenotypic effects of each gene associated with a complex trait are relatively small (Yano and Sasaki, 1997). QTL analysis involves selecting and hybridizing parental lines that differ in one or more quantitative traits and analyzing the segregating progeny to link the QTL to known DNA markers. Chromosomal QTL regions often are quite large and can include many open reading frames. This situation can exacerbate "linkage drag" in the application of QTL analysis to plant breeding or introgression into elite germplasm of undesirable characters that are linked to a desirable QTL (Tanksley

and Nelson, 1996). Thus, a principal objective of QTL analysis is defining QTLs to narrow chromosomal regions, ultimately leading to the identification and cloning of single genes that constitute major QTLs.

The concepts for detecting QTLs were developed more than 75 years ago (Sax, 1923), and in recent years the availability of DNA markers and linkage maps has led to considerable progress in QTL mapping in plants and animals (Lander and Botstein, 1989; Paterson et al., 1989). Plant QTLs that have been cloned include maize *teosinte branched1*, which exerts major effects on plant morphology (Doebley et al., 1997), and tomato *fw2.2*, which controls tomato fruit size (Frary et al., 2000). Nonetheless, the molecular identification of QTLs is still in its infancy. In this issue of *The Plant Cell*, Yano et al. (pp. 2473–2483) describe the use of fine-scale, high-resolution mapping to identify a single gene corresponding to *Hd1*, a major QTL controlling heading date in rice. The *Hd1* gene is a homolog of *CONSTANS* (*CO*) in Arabidopsis, which functions in the photoperiodic control of flowering in this long-day plant (Putterill et al., 1995). This report marks the first demonstration of a QTL in a monocot corresponding to a known mutation in Arabidopsis and only the second flowering-time gene to be cloned from a monocot. The first was the *INDETERMINATE* gene of maize (Colasanti et al., 1998), which has no known ortholog in Arabidopsis. Furthermore, the work of Yano and colleagues shows that similar genes (i.e., putative orthologs) are involved in controlling the daylength response of flowering in short-day (rice) and long-day (Arabidopsis) plants.

THE CIRCADIAN CLOCK AND PHOTOPERIODIC CONTROL OF FLOWERING

The measurement of daylength in plants is controlled by the circadian clock, which controls circadian rhythms in gene expression and behavior that have been widely observed in eukaryotes, including insects, fungi, plants, and vertebrates. Circadian rhythms are 24-hr oscillations in gene expression and/or behavior that are characterized by persistence in the absence of external cues and entrainment by both light and temperature (Kreps and Simon, 1997). The eukaryotic circadian clock has three general components: a "central oscillator" that creates periodicity, inputs that synchronize or entrain the oscillator to the prevailing day–night cycle, and outputs that are the resulting rhythms in gene expression and behavior (Dunlap, 1999; Samach and Coupland, 2000). Four genes in Arabidopsis have been described that are proposed to act within or in close association with the central oscillator: *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CIRCADIAN CLOCK ASSOCIATED1* (*CCA1*), which encode similar MYB proteins, *EARLY FLOWERING3* (*ELF3*), which encodes a novel protein, and *TIMING OF CAB1* (*TOC1*). *TOC1* was recently cloned and identified as an autoregulatory response regulator homolog (Strayer et al., 2000). Mutational analyses, overexpression studies, and mRNA expression patterns of these genes suggest that they perform functions central to the regulation of circadian rhythms (Samach and Coupland, 2000).

The central oscillator is synchronized to the day–night cycle by the

IN THIS ISSUE

action of photoreceptors, namely, the cryptochromes (blue light receptors) and the phytochromes (red light receptors). CRY2 and PHYA may have a primary role in the promotion of flowering in *Arabidopsis* under long-day conditions (Samach and Coupland, 2000). Phytochromes also have been found to play an important role in the photoperiodic control of flowering in rice (Izawa et al., 2000). Somers et al. (1998) examined various *phy* and *cry* mutants of *Arabidopsis* and concluded that photoreceptor diversity and redundancy are key features in the photoperiodic control of the circadian clock in higher plants. A diversity of photoreceptors that cover a wide range of fluence rates and spectral qualities may ensure accurate clock entrainment amid highly variable day-to-day light conditions (Somers et al., 1998). Temperature is another important input signal that affects clock entrainment (Kreps and Simon, 1997).

Flowering time appears to be controlled by a large number of genes. A great many flowering-time mutants (Koornneef et al., 1998) and QTLs (Kuittinen et al., 1997) have been described in *Arabidopsis*, and numerous QTLs for heading date also have been reported in rice (Yano and Sasaki, 1997). Despite the large number of genes involved, the two genes *CO* and *GIGANTEA* (*G1*) exert major control over the promotion of flowering under long days in *Arabidopsis*. These two genes are believed to act immediately downstream of the central oscillator and upstream of numerous other flowering-time genes whose activities they regulate (Nilsson et al., 1998; Samach and Coupland, 2000). They do not appear to act within the central oscillator, because mutations in these genes affect flowering time (and expression of genes downstream in the long day pathway) but not circadian rhythms in general (although *G1* influences *CCA1* expression and is believed to exert some degree of "backward" control on

clock function; Samach and Coupland, 2000). *G1*, originally described as a putative membrane protein (Fowler et al., 1999), was recently determined to be a nuclear protein involved in phytochrome signaling (Huq et al., 2000). *G1* is believed to function upstream of *CO*, because the late-flowering phenotype of *gi* mutants is corrected by *CO* overexpression (Fowler et al., 1999). A putative *G1* ortholog exists in rice, based on the similarity of the predicted *G1* amino acid sequence and rice expressed sequence tag sequences corresponding to a single rice gene. *CO* encodes a protein showing similarity to GATA1-type zinc finger transcription factors (Putterill et al., 1995). A family of *CO*-like regulatory or putative regulatory genes has been identified in *Arabidopsis* and other species (Lagercrantz and Axelsson, 2000), members of which may function as transcriptional activators through DNA binding or protein-protein interactions.

CO AND Hd1 FUNCTION AND LONG-DAY VERSUS SHORT-DAY PLANTS

Flowering in the *Arabidopsis co* mutant occurs later than in the wild type under long days but is unaffected relative to the wild type under short days. In wild-type plants, *CO* mRNA is more abundant in long-day-grown than in short-day-grown seedlings, and overexpression of *CO* can cause early flowering under short days (Putterill et al., 1995). Thus, *CO* appears to be upregulated by long days (or short nights) and to function in the promotion of flowering, perhaps through the activation of floral meristem identity genes such as *LEAFY* (*LFY*) (Simon et al., 1996; Nilsson et al., 1998). Samach et al. (2000) have shown that early target genes of *CO* include *SOC1* and *FT*, which activate floral meristem identity genes (*LFY* in the case of *SOC1*), and genes in the proline and ethylene biosynthetic pathways,

which affect stem elongation and flowering, respectively.

Hd1 encodes a GATA1-type protein that exhibits a high degree of similarity to *CO* in the zinc finger domain and the C-terminal region (Yano et al., 2000). Yamamoto et al. (1998) identified the *Hd1* QTL, along with two other QTLs controlling heading date (*Hd2* and *Hd3*), as single Mendelian factors by crossing two rice varieties, Nipponbare (ecotype *japonica*) and Kasalath (ecotype *indica*), that differ in heading date. Using a combination of self-pollination of progeny and backcrossing to the Nipponbare parental line, plants were obtained that were homozygous for the recessive Kasalath allele at the *Hd1* locus in a Nipponbare background (Yamamoto et al., 1998); such plants were found to be extremely early heading under field conditions (which were similar to long days in growth chamber experiments). Cloning and sequence analysis of *Hd1* by Yano et al. (2000), along with functional complementation experiments, confirmed that Nipponbare carries the functional *Hd1* allele. The recessive Kasalath allele *hd1* was found to contain numerous deletions and one insertion in the coding region compared with the Nipponbare allele. Experiments with various rice lines that are homozygous for either the functional *Hd1* or the mutant *hd1* allele showed that the presence of a functional *Hd1* allele was associated with early heading under short days but, interestingly, significantly later heading under long days. Also, *Hd1* expression appears to be unaffected by daylength, although this tentative conclusion needs to be confirmed with more detailed expression analyses (Yano et al., 2000). Thus, unlike *CO* in *Arabidopsis*, *Hd1* appears to be bifunctional in rice, acting to promote heading under short days and to inhibit it under long days (Lin et al., 2000; Yano et al., 2000).

What distinguishes long-day plants from short-day plants at the molecular level? It is unlikely that the answer lies

IN THIS ISSUE

with a single gene. However, further characterization of the functional differences between *CO* and *Hd1* should bring us closer to understanding the fundamental nature of daylength measurement and the photoperiodic control of flowering in higher plants. Characterization and molecular identification of the putative *Gl* ortholog in rice also should prove to be highly illuminating in this regard.

Nancy A. Eckardt
News and Reviews Editor

REFERENCES

- Colasanti, J., Yuan, Z., and Sundaresan, V. (1998). The indeterminate gene encodes a zinc finger protein and regulates a leaf-generated signal required for the transition to flowering in maize. *Cell* **93**, 593–603.
- Doebley, J., Stec, A., and Hubbard, L. (1997). The evolution of apical dominance in maize. *Nature* **386**, 485–488.
- Dunlap, J.C. (1999). Molecular bases for circadian clocks. *Cell* **96**, 271–290.
- Fowler, S., Lee, K., Onouchi, H., Samach, A., Richardson, K., Morris, B., Coupland, G., and Putterill, J. (1999). *GIGANTEA*: A circadian clock-controlled gene that regulates photoperiodic flowering in *Arabidopsis* and encodes a protein with several possible membrane-spanning domains. *EMBO J.* **18**, 4679–4688.
- Frary, A., Nesbitt, T.C., Frary, A., Grandillo, S., van der Knapp, E., Cong, B., Liu, J., Meller, J., Elber, R., Alpert, K.B., and Tanksley, S.D. (2000). *fw2.2*: A quantitative trait locus key to the evolution of tomato fruit size. *Science* **289**, 85–88.
- Garner, W.W., and Allard, H.H. (1920). Effects of the relative length of night and day and other factors of the environment on growth and reproduction in plants. *J. Agric. Res.* **18**, 553–606.
- Huq, E., Tepperman, J.M., and Quail, P.H. (2000). *GIGANTEA* is a nuclear protein involved in phytochrome signaling in *Arabidopsis*. *Proc. Natl. Acad. Sci. USA* **97**, 9789–9794.
- Izawa, T., Oikawa, T., Tokutomi, S., Okuno, K., and Shimamoto, K. (2000). Phytochromes confer the photoperiodic control of flowering in rice (a short-day plant). *Plant J.* **22**, 391–399.
- Koornneef, M., Alonso-Blanco, C., Blankestijn-de Vries, H., Hanhart, C.J., and Peeters, A.J.M. (1998). Genetic interactions among late-flowering mutants of *Arabidopsis*. *Genetics* **148**, 885–892.
- Kreps, J.A., and Simon, A.E. (1997). Environmental and genetic effects on circadian clock-regulated gene expression in *Arabidopsis*. *Plant Cell* **9**, 297–304.
- Kuittinen, H., Sillanpää, M.J., and Savolainen, O. (1997). Genetic basis of adaptation: Flowering time in *Arabidopsis thaliana*. *Theor. Appl. Genet.* **95**, 573–583.
- Lagercrantz, U., and Axelsson, T. (2000). Rapid evolution of the family of CONSTANS LIKE genes in plants. *Mol. Biol. Evol.* **17**, 1499–1507.
- Lander, E.S., and Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics* **121**, 185–199.
- Li, Z., Pinson, S.R.M., Stansel, J.W., and Park, W.D. (1995). Identification of quantitative trait loci (QTLs) for heading date and plant height in cultivated rice (*Oryza sativa* L.). *Theor. Appl. Genet.* **91**, 374–381.
- Lin, H.X., Yamamoto, T., Sasaki, T., and Yano, M. (2000). Characterization and detection of epistatic interactions of three QTLs, *Hd1*, *Hd2* and *Hd3*, controlling heading date in rice using nearly isogenic lines. *Theor. Appl. Genet.* **101**, 1021–1028.
- Nilsson, O., Lee, I., Blásquez, M.A., and Weigel, D. (1998). Flowering-time genes modulate the response to *LEAFY* activity. *Genetics* **150**, 403–410.
- Paterson, A.H., Lander, E.S., Hewitt, J.D., Peterson, S., Lincoln, S.E., and Tanksley, S.D. (1989). Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphisms. *Nature* **335**, 721–726.
- Poonyarit, M., Mackill, D.J., and Vergara, B.S. (1989). Genetics of photoperiod sensitivity and critical daylength in rice. *Crop Sci.* **29**, 647–652.
- Putterill, J., Robson, F., Lee, K., Simon, R., and Coupland, G. (1995). The *CONSTANS* gene of *Arabidopsis* promotes flowering and encodes a protein showing similarities to zinc finger transcription factors. *Cell* **80**, 847–857.
- Samach, A., and Coupland, G. (2000). Time measurement and the control of flowering in plants. *Bioessays* **22**, 38–47.
- Samach, A., Onouchi, H., Gold, S.E., Ditta, G.S., Schwarz-Sommer, Z., Yanofsky, M.F., and Coupland, G. (2000). Distinct roles of CONSTANS target genes in reproductive development in *Arabidopsis*. *Science* **288**, 1613–1616.
- Sax, K. (1923). The association of size differences with seed-coat pattern and pigmentation in *Phaseolus vulgaris*. *Genetics* **8**, 552–560.
- Simon, R., Igeno, M.I., and Coupland, G. (1996). Activation of floral meristem identity genes in *Arabidopsis*. *Nature* **384**, 59–62.
- Somers, D.E., Devlin, P.F., and Kay, S.A. (1998). Phytochromes and cryptochromes in the entrainment of the *Arabidopsis* circadian clock. *Science* **282**, 1488–1490.
- Strayer, C., Oyama, T., Schultz, T.F., Raman, R., Somers, D.E., Más, P., Panda, S., Kreps, J.A., and Kay, S.A. (2000). Cloning of the *Arabidopsis* clock gene *TOC1*, an autoregulatory response regulator homolog. *Science* **289**, 768–771.
- Tanksley, S.D., and Nelson, J.C. (1996). Advanced backcross QTL analysis: A method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet.* **92**, 191–203.
- Yamamoto, T., Kuboki, Y., Lin, S.Y., Sasaki, T., and Yano, M. (1998). Fine mapping of quantitative trait loci *Hd-1*, *Hd-2*, and *Hd-3*, controlling heading date of rice, as single Mendelian factors. *Theor. Appl. Genet.* **97**, 37–44.
- Yano, M., and Sasaki, T. (1997). Genetic and molecular dissection of quantitative traits in rice. *Plant Mol. Biol.* **35**, 145–153.
- Yano, M., Katayose, Y., Ashikari, M., Yamanouchi, U., Monna, L., Fuse, T., Baba, T., Yamamoto, K., Umehara, Y., Nagamura, Y., and Sasaki, T. (2000). *Hd1*, a major photoperiod sensitivity QTL in rice, is closely related to the *Arabidopsis* flowering time gene *CONSTANS*. *Plant Cell* **12**, 2473–2483.

MEETING REPORT

Arabidopsis Research 2000

The 11th International Conference on Arabidopsis Research, organized by Rick Amasino, Jeffery Dangel, Mary Lou Guerinot, and Detlef Weigel, was held June 24 to 28, 2000, at the University of Wisconsin, Madison. The conference was attended by more than 900 participants and included a large number of students and postdocs. As in past years, sessions and workshops were devoted to a variety of topics ranging from natural variation to genomics to cell biology. Research findings presented by the more than 50 speakers illustrated why this compact weed has made a major impact on our understanding of the mechanisms by which plants grow, reproduce, and respond to the environment. In recent years, reports have been found in almost every issue of *The Plant Cell* that offer testimony to the usefulness of the Arabidopsis model system for speeding the progress of gene discovery and functional characterization in other species, monocots as well as dicots (see this issue's IN THIS ISSUE for just one example). Arabidopsis will be in the spotlight again in upcoming months, after the unveiling of the complete genome sequence this month. This report highlights selected talks and posters that were presented at the June 2000 conference. Meeting abstracts are available at http://www.wisc.edu/union/info/conf/arabidopsis/Arab_abs.pdf.

SIGNAL TRANSDUCTION OF HORMONES

Brassinosteroids (BR) make up a distinctive class of plant sterols essential for plant development. Genetic and molecular approaches have identified

a putative BR receptor (BRI1) and a few BR-regulated genes. However, the signal transduction events between hormone perception and cellular responses remain to be uncovered. Jianming Li (University of Michigan, Ann Arbor) discussed plant steroid signaling and reported the isolation of additional BR-insensitive mutants, such as *bin2*. The *BIN2* gene encodes a cytoplasmic serine/threonine kinase that may play a regulatory role in BR signaling downstream of BRI1. Kathrin Schrick (University of Tübingen, Germany) and Jun-Xian He (Ohio State University, Columbus) provided functional evidence that the early embryogenesis mutant *fackel* (*fk*) is defective in sterol biosynthesis (see also Cell Biology below). Interestingly, exogenous BR does not rescue the *fk* phenotype, which indicates the importance of sterols other than BRs in plant development (Jang et al., 2000; Schrick et al., 2000).

Cytokinins are the least well understood of the classic plant hormones. Joe Kieber (University of North Carolina, Chapel Hill) reported the cloning of a number of primary cytokinin response genes that are rapidly and specifically induced by the hormone. Two of those early genes belong to the large family of Arabidopsis *response regulators* (*ARR*), which are implicated in two-component phosphorelay transduction of various plant signals. The cytokinin-inducible *ARR* genes are providing excellent tools for the study of cytokinin signal transduction. In a genetic approach to cytokinin signaling, Minoru Kubo (Osaka University, Japan) screened for *cytokinin-hyper-sensitive* (*ckh*) mutants, and two recessive mutations were identified in a tissue culture system. The evidence suggests that *CKH1* and *CKH2* func-

tion as negative regulators of cytokinin signaling in Arabidopsis. Map-based cloning revealed that *CKH1* encodes a TATA box binding protein-associated factor.

Early responding genes have proven useful for elucidating the molecular mechanisms of auxin action. The *Aux/IAA* gene class codes for short-lived nuclear transcription factors. Gain-of-function mutations in *Aux/IAA* genes lead to morphological aberrations consistent with enhanced auxin responses and induced ectopic light responses in dark-grown seedlings. Steffen Abel (University of California, Davis) reported that *Aux/IAA* proteins interact with recombinant oat phytochrome A *in vitro* and are substrates for its protein kinase activity. *In vivo* studies showed that at least some of the *Aux/IAA* proteins are phosphoproteins and that the gain-of-function mutations cause protein accumulation. If verified *in vivo*, phosphorylation of *Aux/IAA* proteins by phytochrome may provide one molecular mechanism for the integration of auxin and light signaling in plant development.

Ethylene is arguably the best understood plant hormone. The simple and hormone-specific "triple response" phenotype has allowed a swift and forceful genetic determination of its signaling pathway. Using the typical triple response assay and nonsaturating concentrations of the precursor to ethylene, Anna Stepanova (University of Pennsylvania, Philadelphia) reported the identification of a set of new ethylene-insensitive mutants. These plants are defective in their responses to low ethylene concentrations but respond normally to saturating levels. The isolated mutations affect ethylene receptor genes and new loci important to ethylene signaling.

MEETING REPORT

LIGHT-INDUCED SIGNALING

In the past year, tremendous progress has been made in understanding the light control of plant development. Emmanuel Liscum (University of Missouri, Columbia) presented recent progress on the blue light-mediated phototropic response. Several downstream components of the blue light photoreceptor phototropin (formerly known as NPH1; Christie et al., 1999) have been identified. One of the components, NPH3, was predicted to act closely with phototropin at the genetic level. Molecular cloning revealed that it is a novel protein exhibiting light-dependent interaction with phototropin (Motchoulski and Liscum, 1999). Interestingly, NPH3 appears to work at low fluence rate conditions, whereas another closely related gene family member, RPT2 (Sakai et al., 2000), appears to function at high fluence rate conditions. Another downstream component, NPH4, was speculated to be involved in regulating differential growth and differential auxin distribution based on genetic studies. Recently, it was cloned by Liscum's group and revealed to be the auxin-responsive transcription activator ARF7 (Harper et al., 2000). This result clearly established that regulated gene activation within the nucleus is essential for the phototropic response (or at least the differential growth aspect).

Tatsuya Sakai of the Okada laboratory (Kyoto University, Japan) reported important progress in the search for blue light photoreceptors. This group used a reverse genetics approach to obtain a T-DNA insertion mutant of *NPL1*, a phototropin-like gene. Through examination of all possible blue light responses, they determined that *NPL1* is responsible for chloroplast movement in *Arabidopsis* leaf cells, a high-fluence blue light response. Further studies confirmed that *NPL1* is a photoreceptor for blue light and responsible for high-fluence blue light-induced chloroplast

movement. Although this mutant by itself has no detectable defect in phototropism, plants with mutations in *np1* and phototropin have an enhanced defect in phototropism, implying a partial role for *NPL1* in phototropism as well.

A conceptual breakthrough in phytochrome signaling was reported recently by Peter Quail's laboratory (University of California, Berkeley). After light-activated nuclear import, phytochrome can bind directly to the transcription factor PIF3 that is bound to a light-responsive promoter element and thus alter the expression of light-responsive genes (Martinez-Garcia et al., 2000). Christian Fankhauser (University of Geneva, Switzerland) formerly of the Chory laboratory (Salk Institute, San Diego, CA) presented the cloning of a new gene, *RSF1*, that has a high homology to *PIF3*. The *rsf1* mutant was identified by its long hypocotyl phenotype in far-red light, suggesting that *RSF1* acts in phytochrome A signaling. Indeed, this gene seems to be a key player in phytochrome A signaling, because it was also independently identified and characterized by Peter Quail's group (P. Quail, personal communication).

Progress was also reported regarding the COP/DET/FUS group of proteins, which act downstream of multiple photoreceptors to mediate the light control of development. One new concept put forward by the Deng laboratory (Yale University, New Haven, CT) is that these proteins may be novel regulators of the ubiquitin-proteasome pathway. In particular, it was hypothesized that COP1 may act as an E3 ligase that targets photomorphogenesis-promoting factors for degradation (Osterlund et al., 2000). Although the COP9 signalosome is essential for COP1-mediated protein degradation, it seems to play a pleiotropic role in that partial loss-of-function antisense lines exhibited a large variety of developmental defects. In addition to the COP9 signalosome, the Chory laboratory also reported that DET1 protein is present in

a complex in vivo. Clearly, more work is required to understand the biochemical activities of the COP/DET/FUS proteins or protein complexes.

REPRODUCTIVE DEVELOPMENT

In the ABC model of flower development, the prototypical C function gene *AGAMOUS* (*AG*) plays a role in the specification of stamen and carpel identity. Two other C function genes, *HUA1* and *HUA2*, which appear to perform functions similar to *AG*, have been identified (Chen and Meyerowitz, 1999). Mutations in both *HUA1* and *HUA2* only weakly affect stamen and carpel phenotypes but strongly enhance *ag* mutations. Xuemei Chen (Rutgers University, New Brunswick, NJ) described enhancers of *hua1 hua2* double mutants, designated *HEN1* and *HEN2*. In this digenic mutant background, the *hen* mutations cause the homeotic transformation of stamens into petal-like and sepal-like organs, with only minor effects on carpels. This result implicates the *HEN* genes in the specification of third whorl floral organ identity, possibly by acting in concert with or downstream of B and C function genes. Cloning of the *HEN1* gene showed that it encodes a novel protein.

The role of the *NOZZLE* (*NZZ*) gene in ovule development was discussed by Sureshkumar Balasubramanian from Kay Schneitz's laboratory (University of Zurich, Switzerland). *nzz* mutants exhibit both female and male sterility due to the severe reduction in the nucellus and pollen sacs, as shown by the absence of megaspore and pollen mother cells (Schiefthaler et al., 1999). The *NZZ* gene, also known as *SPOROCTYLESS*, has been cloned and shown to encode a putative nuclear protein of unknown function (Schiefthaler et al., 1999; Yang et al., 1999). The authors reported digenic mutant analyses and in situ hybridization experiments sug-

MEETING REPORT

gesting that *NZZ* is a transcriptional repressor of genes involved in ovule formation. Specifically, *NZZ* interacts negatively with *AINTEGUMENTA*, a gene that plays a role in cell proliferation, and *BEL1* and *INNER NO OUTER*, genes involved in proximal-distal and adaxial-abaxial patterning of the ovule, respectively. These interactions suggest that *NZZ* couples pattern formation and growth during ovule development.

Cory Christensen of Gary Drews's laboratory (University of Utah, Salt Lake City) described a genetic screen to identify genes involved in female gametophyte development and function. Female gametophyte mutants, identified on the basis of reduced seed set and reduced transmission of T-DNA causing the mutation, constituted ~0.8% of T-DNA mutagenized lines (Drews et al., 1998). Mutants were grouped according to the stage at which female gametophyte development was arrested, and the identity of the disrupted gene was established by analysis of plant DNA flanking the mutagenic T-DNA. Christensen discussed two genes corresponding to mutations that cause defects in the fusion of polar nuclei of the central cell of the female gametophyte (Christensen et al., 1998). One gene encodes a DnaJ homolog and the other encodes a protein with sequence similarity to NSF, an AAA ATPase. Both classes of genes play a role in nuclear membrane fusion in yeast.

FLORAL INDUCTION

In recent years, numerous flowering-time genes have been isolated. These genes are essential tools to test models for the control of flowering time that have been proposed mainly based on genetic interactions. According to mutant analysis, flowering-time genes can be grouped into three main floral inductive pathways that promote the transition to reproductive development: a

daylength-dependent pathway active only under long days, a daylength-independent (or autonomous) pathway active under both long days and short days, and a gibberellin-dependent pathway (Koornneef et al., 1998). Molecular data presented at the meeting revealed the connections between the different pathways, allowing an understanding of how the plant integrates environmental and endogenous information to trigger flowering at the appropriate time.

According to newly identified molecular targets in the long day-dependent pathway, signaling seems to branch downstream of *CONSTANS* (*CO*); one pathway, mediated by *SOC1* (*AGL20*), leads to the activation of *LEAFY* (*LFY*) transcription (Onouchi et al., 2000; Samach et al., 2000), and another, mediated by *FT*, leads to the activation of *APETALA1* (*AP1*; Ruiz-García et al., 1997; Kardailsky et al., 1999; Kobayashi et al., 1999). Interestingly, Takashi Araki (Kyoto University) reported that *FT* expression was downregulated in mutants that define the daylength-independent pathway, such as *fca* and *fve*, establishing a molecular mechanism for the interaction between both pathways and explaining why *fca* mutants appear as suppressors of *CO* overexpression. S. Michaels (University of Wisconsin, Madison) reported that this interaction is most likely mediated by *FLC*, because an *flc* loss-of-function mutation is epistatic to mutations in the daylength-independent pathway. A tighter connection between the long day and the autonomous pathways was established by Ilha Lee (Seoul National University, South Korea) with the finding that *SOC1* overexpression suppressed the requirement for vernalization in plants bearing a late allele of *FRIGIDA*, a gene that interacts with *FLC* downstream of the daylength-independent pathway (Lee et al., 1994). Furthermore, *SOC1* expression also was reduced in the same set of mutants that affect *FT* expression, and there was an

inverse correlation between *FLC* and *SOC1* mRNA levels.

Two other reports addressed the question of integration of floral inductive pathways using a different strategy. Nobumasa Yoshida (Mitsui Chemicals, Tokyo, Japan) reported on the cloning of *EMF2*, which together with *EMF1* has been assumed to play a central role as a repressor of the transition to the reproductive phase. This is based on the very early flowering—right after germination—of *emf* mutant plants (Sung et al., 1992; Chen et al., 1997) and the fact that *emf* mutations appear to be epistatic to all tested flowering-time mutations (Haung and Yang, 1998). According to this view, all floral promotive cues would merge on the *EMF* gene products to modulate their repressing activity. *EMF2* encodes a zinc finger protein similar to *FIS2*, a repressor of seed development (Luo et al., 1999). Unfortunately, attempts to overexpress the *EMF* genes, and thus determine whether relative levels of EMFs modulate flowering time, have been unsuccessful. Finally, Miguel Blázquez (Salk Institute) reported that elimination of a *cis*-acting sequence in the *LFY* promoter abolished the regulation of *LFY* expression by gibberellins but not by the long day pathway. This result suggests that the promoters of floral meristem identity genes, which link floral induction with floral initiation, may act as integrators of at least these two pathways.

Together, the data presented show that we can now link the daylength-independent pathway to the long day pathway at the molecular level. Furthermore, the gibberellin-dependent pathway and the long day pathway integrate at the level of the *LFY* promoter. However, because we still lack information on the nature of the *trans*-acting factors that interact with the promoters of the floral meristem identity genes (except for the fact that *LFY* binds to the *AP1* promoter; Busch et al., 1999; Wagner et al., 1999), we have

MEETING REPORT

ignored the details of the connection between the flowering-time genes and floral initiation. This gap in the signaling pathway will be a critical one to fill in the future.

CELL BIOLOGY

Progress in understanding the control of cellular organization and growth using traditional biochemical and cell biological methods has been slow. The recent use of Arabidopsis genetics shows the great potential for identifying regulatory genes and eventually discovering the mechanisms by which plant cells regulate cellular organization. Dan Szymanski (Purdue University, Lafayette, IN) described the use of leaf trichomes as a powerful single cell system to study the mechanisms of actin-dependent growth in plant cells. The "distorted" group of trichome mutants has an altered actin organization compared with the wild type, and the shape defects in distorted mutants are phenocopied by agents that disrupt actin organization. One of the mutants has been identified as a seedling-lethal mutation in the *SPIKE1* gene that causes widespread defects in epidermal cell and tissue organization. *SPIKE1* is an excellent candidate gene for integrating extracellular information with cytoskeletal organization. It is predicted to be an integral plasma membrane protein with several membrane-spanning domains. A putative cytoplasmic domain of *SPIKE1* shares amino acid identity with the DOCK180 family of proteins that locally regulates actin organization. Geoffrey Wasteneys (Australian National University, Canberra) described work on the phenotypic and genetic analysis of the temperature-sensitive *mor1* (microtubule organization) mutant, which is providing new insight into microtubule organization and cell shape regulation. Cortical microtubules are disrupted at 29°C in *mor1*,

with morphological consequences including left-handed organ twisting, loss of growth anisotropy, and loss of fertility. Curiously, cellulose alignment is apparently unaffected in the mutant, and double mutant analysis with the cellulose-deficient *rsw1* corroborates the suggestion that microtubules influence growth anisotropy by mechanisms other than cellulose alignment. Double mutant analysis also indicates that MOR1 acts independently of the putative microtubule organization gene BOT1, that *mor1* overrides the right-handed spiraling mutant *spr1*, and that MOR1 is fully epistatic to SPR2/TORTIFOLIA.

Our understanding of the role of sterols in cell division and cell expansion in both embryonic and postembryonic development in plants has taken a significant step forward with the cloning of the *FACKEL* (*Fk*) gene by both Gerd Jurgens's group (University of Tübingen) and Jyan-Chyun Jang's group (Ohio State University) (Jang et al., 2000; Schrick et al., 2000). *fk* mutants, first identified in a seedling screen for pattern formation mutants, display cell division and expansion defects in embryogenesis as well as patterning defects, as indicated by misplaced or multiple shoot meristems. The *Fk* gene encodes a sterol C-14 reductase, as suggested by sequence similarity to known sterol reductases, including the vertebrate lamin B receptor and *Saccharomyces cerevisiae* ERG24, and by complementation of the corresponding yeast mutant. In addition, gas chromatography-mass spectrometry analysis of *fk* mutants indicates the accumulation of sterol biosynthetic intermediates at the C-14 reductase step. Kathrin Schrick from the Tübingen group also described the *cephalopod* (*cph*) mutant, which shows similar defects to *fk*, with the corresponding gene encoding a putative sterol C-24 methyltransferase, an enzyme that acts in the same branch of the sterol biosynthesis pathway as the *Fk* C-14 reductase. Both *Fk* and *CPH* genes act upstream of the

BR-specific branch. Because BR dwarf mutants do not show defects in embryogenesis, the findings suggest that sterols other than BR may have structural roles and/or may act as sterol signals during embryogenesis.

Insight into the mechanism by which environmental signals regulate plant size was presented by Jian Hua from the Fink laboratory (Whitehead Institute, Cambridge, MA). The *bonsai* (*bon*) mutant has very small, curled leaves and short, thin stems due to a reduction in both cell size and cell number. Overexpression of the *BON* gene using the cauliflower mosaic virus 35S promoter results in plants with larger leaves and thicker stems. The *BON* gene encodes a calcium-dependent phospholipid binding protein with sequence homology with a family of copine proteins found in human, worm, and *Paramecium*. It has been speculated that these proteins are involved in vesicle fusion or membrane trafficking, and Hua proposed that *BON* regulates cell expansion and division at low temperature by regulating membrane trafficking.

The use of green fluorescent protein (GFP) as a tool to address problems in plant cell biology was highlighted by several exciting oral and poster presentations. Naohiro Kato (Rutgers University) used GFP as an *in vivo* tag of genomic DNA to visualize relative locations of chromatin sites. To achieve this, tandem arrays of the lac operator site were inserted in the genome. Using an inducible expression system and a plant-optimized GFP-LacI fusion protein, the tagged sites (lac operator arrays) were detected as bright spots in nuclei. Three-dimensional images and video displays of tagged sites in nuclei of stomatal cells were presented. A high throughput approach to examining subcellular organization by producing and imaging large numbers of plants that express random GFP::cDNA fusions was presented by Sean Cutler and David Ehrhardt from Chris Somerville's group (Carnegie Institute of Washing-

MEETING REPORT

ton, Stanford, CA) (Cutler et al., 2000). Many unique dynamic cellular processes have been uncovered with this approach. For example, a fusion between GFP and the enzyme nitrilase 1 was found to redistribute from the cytoplasm into granular structures minutes after the delivery of a wound stimulus. These and other observations suggest that the rapid formation of granules reveals a novel, wound-inducible signal pathway and implicates nitrilase in the wound response.

SENESCENCE AND ABSCISSION

A number of presentations highlighted important contributions from work with Arabidopsis to our understanding of the genetic control of the biochemical and physiological events that lead to senescence and abscission. Hong Gil Nam (Pohang University, Korea) reported on the isolation of several mutants that showed delayed leaf senescence. The cloning of one of these *ore* mutants (for *oresara*, meaning “long living” in Korean) revealed that it is disrupted in a gene encoding an F-box protein, which suggests a role for the proteasome complex in regulating senescence. Nam also reported on the isolation and expression patterns of more than 40 senescence upregulated clones. The sequence identities and phenotypes of antisense transgenic plants suggested that some of them are regulatory genes.

Sara Patterson (University of Wisconsin) is using both classic and reverse genetic approaches to identify mutants that regulate floral organ abscission and cell separation. She presented work on the identification and analysis of six novel delayed floral organ abscission mutants (*dab1* to *dab6*) and an ectopic cell separation mutant, *tfa1* (for things fall apart), in which cells in the hypocotyl and cotyledon tissues slough off from the main body of the plant.

Silke Robatzek (Max Planck Institute, Cologne, Germany) presented work on WRKY6, a member of the Arabidopsis WRKY superfamily of plant-specific transcription factors. WRKY6 was identified as a regulatory component of several cell death-related processes, including leaf senescence. Strong WRKY6 expression, mediated by distinct regions of the WRKY6 promoter, was observed in response to pathogen attack, in floral organ abscission zones, and during the early to middle stages of leaf senescence. Interestingly, WRKY6 knockout mutants showed altered PR1 gene expression during leaf senescence. These and other data suggest that WRKY6 plays a central role during cell death determination.

ATHB-1 is an Arabidopsis transcription factor belonging to the homeobox-leucine zipper family. Takuya Muramoto (Kyoto University) presented work on target gene analysis of ATHB-1 using a glucocorticoid fusion protein system in transgenic Arabidopsis that is expected to recognize target genes for ATHB-1 and transactivate them depending on the presence of glucocorticoid. Using this approach, they have identified four genes encoding proteins homologous with amino acid transporters. This finding suggests that ATHB-1 or HD-Zip I family members may regulate nutrient reallocation in leaf development, including leaf senescence.

PATHOGEN RESPONSE

The progress made in elucidating plant-pathogen interactions using Arabidopsis as a model was reflected by the nearly 90 posters in this section and by the breadth of topics addressed by speakers. Three areas of impact were noteworthy. First, there is emerging interest in using natural populations of Arabidopsis and its pathogens to understand the evolution and dispersal of disease resistance (*R*) genes in the

wild. Martin Kreitman presented work from a joint study by his and Joy Bergelson's laboratories (University of Chicago, Illinois) that indicates that at least the *RPM1*, *RPS2*, and *RPS5* loci are evolving slowly and that resistant and susceptible alleles represent ancient, balanced polymorphisms (Stahl et al., 1999). The Bergelson-Kreitman group argue that their results suggest a selective cost acting on resistant alleles in the host, possibly a consequence of the detrimental effects of residual basal activity, or incomplete shut off, of nucleotide binding site-leucine-rich repeat (NBS-LRR) proteins, which can lead to cell death when activated. In addition, they find that none of these single-copy loci exhibit rapid protein evolution, a common characteristic of complex *R* loci, leading to the controversial conclusion that these loci are not engaged in an “arms race,” as is commonly portrayed in textbooks. An alternative hypothesis is that the polymorphisms at the simple loci analyzed (there are only presence and absence alleles of *RPM1* and *RPS5*) are evolutionarily stable and are not responding to pathogen pressure with rapid evolution or duplication. Analysis of complex *R* loci (Noël et al., 1999) may challenge this model, as suggested by results on the polymorphism in *RPP8*, a locus with copy number variation (McDowell et al., 1998) that paradoxically shows indications of both rapid evolution and ancient alleles (J. Bergelson, personal communication).

Eric Holub (Horticultural Research International, Wellesbourne, UK) presented a summary of his decade-long analysis of the natural coevolved interactions between Arabidopsis and two biotrophic oomycetes, *Albugo candida* (white rust) and *Peronospora parasitica* (downy mildew). Summarizing what we know regarding resistance to these parasites, he suggested that layers of signal transduction loci are recruited to function in conjunction with a variety of specific *R* genes. Moreover, multiple

MEETING REPORT

layers of overlapping defense responses may explain some examples of species-level or so-called non-host resistance (in which all isolates of a given pathogen are recognized by all genotypes of a plant species). Holub described examples of how mutants selected in *Arabidopsis* can be used to reveal genes that provide components of non-host resistance, such as the mutant *eds1*, which is fully susceptible to some isolates of *Albugo* from Brassica. Novel signal pathways are also yet to be defined. *RPP7*, a downy mildew resistance gene that uses a novel defense response (McDowell et al., 2000), requires the action of at least three additional genes, defined by the *edm1*, *edm2*, and *edm3* mutants. Finally, Holub described the characterization of 40 isolates of *Pseudomonas syringae* collected from Brassica. *RPM1* was found to provide resistance to a majority of them, even though it was originally defined using avirulence genes isolated from *P. syringae* collected from soybean and pea as well as Brassica. Thus, non-host resistance is at least partially explained by specific host resistance (Kobayashi et al., 1989).

This message is resonant given recent results from John Turner's laboratory (University of East Anglia, Norwich, UK). His group has isolated a gene controlling resistance in *Arabidopsis* to several isolates of the powdery mildew *Erysiphe* spp pathogens. This gene does not encode a by-now canonical NBS-LRR protein but rather a small protein of unknown function. The function of *RPW8* depends on at least one known component of TIR/NBS/LRR signaling (the *EDS1* gene; Aarts et al., 1998), suggesting that this novel protein is part of a "normal" transduction pathway. That it, and not the NBS-LRR protein, is the evolutionarily polymorphic component, might suggest that it is a cellular target of an *Erysiphe* virulence factor that is guarded by an NBS-LRR. It will be important to determine what other loci are in this pathway and

whether one of them is an NBS-LRR. The Turner group has already performed this screen and is sorting out the putative mutants.

The second main area of interest, as alluded to above, is the characterization of the signaling pathways required for *R* gene function. Roger Innes's laboratory (Indiana University, Bloomington) defined three loci required for the function of the NBS-LRR gene *RPS5* (Warren et al., 1998). They have now isolated one of them, *pbs1*, and shown that it encodes a serine/threonine kinase with homology to the tomato *PTI1* and *PTO* genes. This result is intriguing because *PTO* is the polymorphic resistance gene against certain *P. syringae* strains in tomato and because its activity requires the NBS-LRR protein PRF (Rathjen et al., 1999). In contrast to the tomato system, in which the *PTO* protein interacts directly with the corresponding avirulence protein AvrPto, Innes reported no interaction between PBS1 and the corresponding AvrPphB protein. But, as Innes pointed out, this is a negative yeast two-hybrid result and must be interpreted with caution. These findings beg the question of whether all NBS-LRR proteins require the function of a specific kinase partner, and if so, what is the normal function of that kinase. The identification of mutants defining genes whose function is required for NBS-LRR action, and the cloning of those genes, is opening many new doors. Not least among them is the establishment of genetic relationships between both *R*-dependent responses and subsequent systemic acquired resistance responses. The cumulative message from a group of posters on this topic from several laboratories is that pathogen response is controlled by networks of intersecting pathways and not by linear responses. As with all double mutant analyses, it is important to use null loss-of-function alleles in these studies to allow the cleanest interpretations, and the cloning of several genes with these pheno-

types in the near future will expand on the genetic analyses now under way.

The final area beginning to alter our understanding of plant-pathogen interactions is transcriptional profiling. Three approaches were represented at the meeting. Two talks described the use of microarrays to determine pathogen and signaling responses. Jonathan Anderson (University of Queensland, St. Lucia, Australia) described results from collaboration between the laboratories of John Manners (Commonwealth Scientific and Industrial Research Organization, Australia) and Shauna Somerville (Carnegie Institute of Washington) aimed at understanding the relationship of transcriptional responses after a variety of treatments. These included both local and systemic responses to infection by *Alternaria brassicicola* and signaling induced by treatment with salicylic acid, methyl jasmonate, and ethylene. These mRNAs were hybridized to a 2375-element "defense array" designed at the Somerville laboratory. These large data sets were condensed to reveal a manageable number of reproducible transcriptional changes. Readers of an age to have learned the "new math" in the late 1960s and early 1970s will be happy to hear that the time spent learning set theory was not wasted, because Anderson presaged an auspicious return of the Venn diagram. He demonstrated that very small subsets of genes (approximately five to 20) can be identified whose transcription is commonly altered by defined sets of inductions.

Bob Dietrich (Novartis Agricultural Biotechnology Institute, Research Triangle Park, NC) expanded on this theme by presenting data from a collaboration between his laboratory and that of Jeff Dangl (University of North Carolina). They used 14 treatments related to the induction of systemic acquired resistance. The data were analyzed for genes whose expression was altered in two or more of the 14 systemic acquired resistance-relevant

MEETING REPORT

conditions. The regulon of genes containing the well-known *PR-1* gene was analyzed in detail. Dietrich described the finding of a common transcriptional control element in promoters from the 26 genes in this regulon for which genomic sequence was available (Maleck et al., 2000). This element binds transcriptional regulators of the WRKY class (Eulgem et al., 2000). These data are important because they establish that coregulated genes can share definable *cis* sequences and because they used mutants, as well as responses to infection, to define the set of genes to be analyzed. Indeed, the power of this technology, specifically as used by Dietrich and colleagues, is not so much in the identification of genes per se but in the determination of response pathways.

The future of expression profiling was summarized in two talks by Tong Zhu and Steve Whitham, both from the Novartis Agricultural Discovery Institute (San Diego, CA). Zhu described the design and characterization of the Arabidopsis GENECHIP genome array, which contains probe sets for more than 8000 genes, and his efforts to establish quality control and reproducibility. Zhu developed standardized protocols for sample preparation and used multiple quality control steps to ensure the reproducibility of the results. The data were stunning, with chip-to-chip variation of less than 0.3%, even across different chip lots. Whitham presented his analysis of responses to disease caused by five different compatible virus infections. He identified and categorized roughly 120 genes whose activation is common to these five viral infections, of which 36 are not transcriptionally activated during the onset of bacterial disease. He also described 45 genes repressed by these viral infections. Surprisingly, none of them is repressed during the onset of bacterial disease. These could be targets of viral proteins whose function is to disarm the host resistance response, a notion that Whitham is keen to address.

Finally, several groups are using a variety of differential cloning strategies to identify novel transcripts whose transcription is altered after pathogen attack. This is important because the expressed sequence tag database contains genes expressed in healthy tissue and not those induced under infection conditions. Ramesh Raina and colleagues (Pennsylvania State University, University Park) displayed a poster showing that there are several hundred new expressed sequence tags to be found after either salicylic acid or *RPM1* triggering. The existence of these clones, and of all others like them from different "induced screenings," will be an immense boon not only to gene discovery in host-pathogen interactions but to the annotation of predicted open reading frames in the Arabidopsis genome.

Steffen Abel
University of California
Davis, California

Miguel Blázquez
Universidad Politecnica de
Valencia-C.S.I.C.,
Valencia, Spain

Jeffery Dangl
University of North Carolina
Chapel Hill, North Carolina

Xing Wang Deng
Yale University
New Haven, Connecticut

Ian Graham
University of York
York, England

John Harada
University of California
Davis, California

Jonathan Jones
John Innes Center
Norwich, England

Ove Nilsson
Swedish University of
Agricultural Sciences
Umeå, Sweden

REFERENCES

- Aarts, N., Metz, M., Holub, E., Staskawicz, B.J., Daniels, M.J., and Parker, J.E.** (1998). Different requirements for EDS1 and NDR1 by disease resistance genes define at least two R gene mediated signalling pathways in Arabidopsis. *Proc. Natl. Acad. Sci. USA* **95**, 10306–10311.
- Busch, M.A., Bomblies, K., and Weigel, D.** (1999). Activation of a floral homeotic gene in Arabidopsis. *Science* **285**, 585–587.
- Chen, L., Cheng, J.C., Castle, L., and Sung, Z.R.** (1997). *EMF* genes regulate Arabidopsis inflorescence development. *Plant Cell* **9**, 2011–2024.
- Chen, X., and Meyerowitz, E.M.** (1999). *HUA1* and *HUA2* are two members of the floral homeotic AGAMOUS pathway. *Mol. Cell* **3**, 349–360.
- Christensen, C.A., Subramanian, S., and Drews, G.N.** (1998). Identification of gametophytic mutations affecting female gametophyte development in Arabidopsis. *Dev. Biol.* **202**, 136–151.
- Christie, J.M., Salomon, M., Nozue, K., Wada, M. and Briggs, W.R.** (1999). LOV (light, oxygen, or voltage) domains of the blue-light photoreceptor phototropin (*nph1*): Binding sites for the chromophore flavin mononucleotide. *Proc. Natl. Acad. Sci. USA* **96**, 8779–8783.
- Cutler, S.R., Ehrhardt, D.W., Griffiths, J.S., and Somerville, C.R.** (2000). Random GFP::cDNA fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency. *Proc. Natl. Acad. Sci. USA* **97**, 3718–3723.
- Drews, G.N., Lee, D., and Christensen, C.A.** (1998). Genetic analysis of female gametophyte development and function. *Plant Cell* **10**, 5–17.
- Eulgem, T., Rushton, P.J., Robatzek, S., and Somssich, I.E.** (2000). The WRKY superfamily of plant transcription factors. *Trends Plant Sci.* **5**, 199–206.

MEETING REPORT

- Harper, R.M., Stowe-Evans, E.L., Luesse, D.R., Muto, H., Tatematsu, K., Watahiki, M.K., Yamamoto, K., and Liscum, E.** (2000). The NPH4 locus encodes the auxin response factor ARF7, a conditional regulator of differential growth in aerial Arabidopsis tissue. *Plant Cell* **12**, 757–770.
- Haug, M.D., and Yang, C.H.** (1998). *EMF* genes interact with late-flowering genes to regulate *Arabidopsis* shoot development. *Plant Cell Physiol.* **39**, 382–393.
- Jang, J.C., Fugjioka, S., Tasaka, M., Seto, H., Takatsuto, S., Ishii, A., Aida, M., Yoshida, S., and Sheen, J.** (2000). A critical role of sterols in embryonic patterning and meristem programming revealed by the *fackel* mutants of *Arabidopsis thaliana*. *Genes Dev.* **14**, 1485–1497.
- Kardailsky, I., Shukla, V., Ahn, J.H., Dagenais, N., Christensen, S.K., Nguyen, J.T., Chory, J., Harrison, M.J., and Weigel, D.** (1999). Activation tagging of the floral inducer *FT*. *Science* **286**, 1962–1965.
- Kobayashi, D.Y., Tamaki, S.J., and Keen, N.T.** (1989). Cloned avirulence genes from the tomato pathogen *Pseudomonas syringae* pv. *tomato* confer cultivar specificity on soybean. *Proc. Natl. Acad. Sci. USA* **86**, 157–161.
- Kobayashi, Y., Kaya, H., Goto, K., Iwabuchi, M., and Araki, T.** (1999). A pair of related genes with antagonistic roles in mediating flowering signals. *Science* **286**, 1960–1962.
- Koornneef, M., Alonso-Blanco, C., Peeters, A.J.M., and Soppe, W.** (1998). Genetic control of flowering in Arabidopsis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **49**, 345–370.
- Lee, I., Michaels, S.D., Masshardt, A.S., and Amasino, R.M.** (1994). The late-flowering phenotype of *FRIGIDA* and mutations in *LUMINIDEPENDENS* is suppressed in the Landsberg *erecta* strain of *Arabidopsis*. *Plant J.* **6**, 903–909.
- Luo, M., Bilodeau, P., Koltunow, A., Dennis, E.S., Peacock, W.J., and Chaudhury, A.M.** (1999). Genes controlling fertilization-independent seed development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **96**, 296–301.
- Maleck, K., Levine, A., Eulgem, T., Morgan, A., Schmid, J., Lawton, K., Dangl, J.L., and Dietrich, R.A.** (2000). The transcriptome of Arabidopsis during systemic acquired resistance. *Nat. Genet.* (in press).
- Martinez-Garcia, J.F., Huq, E., and Quail, P.H.** (2000). Direct targeting of light signals to a promoter element-bound transcription factor. *Science* **288**, 859–863.
- McDowell, J.M., Dhandaydham, M., Long, T.A., Aarts, M.G.M., Goff, S., Holub, E.B., and Dangl, J.L.** (1998). Intragenic recombination and diversifying selection contribute to the evolution of downy mildew resistance at the *RPP8* locus of Arabidopsis. *Plant Cell* **10**, 1861–1874.
- McDowell, J.M., Cuzick, A., Can, C., Beynon, J., Dangl, J.L., and Holub, E.B.** (2000). Downy mildew (*Peronospora parasitica*) resistance genes in Arabidopsis vary in functional requirements for *NDR1*, *EDS1*, *NPR1*, and salicylic acid accumulation. *Plant J.* **22**, 523–530.
- Motchoulski, A., and Liscum, E.** (1999). Arabidopsis NPH3: A NPH1 photoreceptor-interacting protein essential for phototropism. *Science* **286**, 961–964.
- Noël, L., Moores, T.L., van der Biezen, E.A., Parniske, M., Daniels, M.J., Parker, J.E., and Jones, J.D.G.** (1999). Pronounced intraspecific haplotype divergence at the *RPP8* complex disease resistance locus of Arabidopsis. *Plant Cell* **11**, 2099–2111.
- Onouchi, H., Igeño, M., Périlleux, C., Graves, K., and Coupland, G.** (2000). Mutagenesis of plants overexpressing *CONSTANS* demonstrates novel interactions among Arabidopsis flowering-time genes. *Plant Cell* **12**, 885–900.
- Osterlund, M.T., Hardtke, C., Wei, N., and Deng, X.W.** (2000). Targeted destabilization of HY5 during light regulated development of Arabidopsis. *Nature* **405**, 462–466.
- Rathjen, J.P., Chang, J.H., Staskawicz, B.J., and Michelson, R.W.** (1999). Constitutively active *Pto* induces a *Prf*-dependent hypersensitive response in the absence of *avrPto*. *EMBO J.* **18**, 3232–3240.
- Ruiz-García, L., Madueño, F., Wilkinson, M., Haughn, G., Salinas, J., and Martínez-Zapater, J.M.** (1997). Different roles of flowering time genes in the activation of floral initiation genes in Arabidopsis. *Plant Cell* **9**, 1921–1934.
- Sakai, T., Wada, T., Ishiguro, S., and Okada, K.** (2000). RPT2: A signal transducer of the phototropic response in Arabidopsis. *Plant Cell* **12**, 225–236.
- Samach, A., Onouchi, H., Gold, S., Ditta, G., Schwarz-Sommer, Z., Yanofsky, M., and Coupland, G.** (2000). Distinct roles of *CONSTANS* target genes in reproductive development of Arabidopsis. *Science* **288**, 1613–1616.
- Schieffhale, U., Balasubramanian, S., Sieber, P., Chevalier, D., Wisman, E., and Schneitz, K.** (1999). Molecular analysis of *NOZZLE*, a gene involved in pattern formation and early sporogenesis during sex organ development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* **96**, 11664–11669.
- Schrack, K., Mayer, U., Horrichs, A., Kuhnt, C., Bellini, C., Dangl, J., Schmidt, J., and Jurgens, G.** (2000). FACKEL is a sterol C-14 reductase required for organized cell division and expansion in Arabidopsis embryogenesis. *Genes Dev.* **14**, 1471–1484.
- Stahl, E.A., Dwyer, G., Mauricio, R., Kreitman, M., and Bergelson, J.** (1999). Dynamics of disease resistance polymorphism at the *RPM1* locus of *Arabidopsis*. *Nature* **400**, 667–671.
- Sung, Z.R., Belachew, A., Shunong, B., and Bertrand-Garcia, R.** (1992). *EMF*, an *Arabidopsis* gene required for vegetative shoot development. *Science* **258**, 1645–1647.
- Wagner, D., Sablowski, R.W.M., and Meyerowitz, E.M.** (1999). Transcriptional activation of *APETALA1* by *LEAFY*. *Science* **285**, 582–584.
- Warren, R.F., Henk, A., Mowery, P., Holub, E., and Innes, R.W.** (1998). A mutation within the leucine-rich repeat domain of the Arabidopsis disease resistance gene *RPS5* partially suppresses multiple bacterial and downy mildew resistance genes. *Plant Cell* **10**, 1439–1452.
- Yang, W.-C., Ye, D., Xu, J., and Sundaresan, V.** (1999). The *SPOROXYTELESS* gene of Arabidopsis is required for initiation of sporogenesis and encodes a novel nuclear protein. *Genes Dev.* **13**, 2108–2117.