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MEETING REPORT

Arabidopsis Research 2001

Arabidopsis researchers from all over the world once again converged on the shores of Lake Mendota in Madison, Wisconsin, from June 23 to 27 for the 12th International Conference on Arabidopsis Research. In this report, we make no attempt to cover the entire meeting; rather, we present summaries of a number of presentations that we hope will convey something of the breadth and depth of the many exciting new discoveries in plant biology in the last year. A complete listing of abstracts can be found at http://www.wisc.edu/union/info/conf/arabidopsis/Abstracts_01.pdf.

It is difficult to speak of "general themes" at a meeting that encompassed so many aspects of plant growth and development. However, at least three topics emerged repeatedly throughout the conference. One of these was the primary role of proteolysis in the regulation of metabolic pathways. Perhaps the best-characterized example is the role of the COP9 signalosome in the regulation of photomorphogenesis (see Responses to the Abiotic Environment below). At this meeting, we also heard about links between proteolysis and hormone signaling (see Inductive Processes) and disease resistance (see Responses to the Biotic Environment). A second major theme was found in the rapid strides being made in understanding signal transduction and signaling networks. The current literature is replete with examples and discussions of "cross-talk" (i.e., interactions) between signaling pathways. We are beginning to realize that it may be more appropriate to think of "signaling networks" than "pathways." Of course, this dawning realization has been accompanied by the development of the necessary tools and techniques to analyze the complex interactions that make up a network. Finally, scattered throughout the meeting were many fine examples of innovative genetic and genomic screens for investigating the functions of genes and proteins.

Steve Henikoff (Fred Hutchinson Cancer Research Center, Seattle, WA) provided excellent examples of the latter topic in his keynote address, in which he emphasized that "necessity is the mother of invention." In other words, basic researchers, struggling with problems in their own laboratories, and not companies, are best equipped to develop new technologies. Henikoff gave two examples from his laboratory. The first was the development of DamID (van Steensel et al., 2001), a technique that arose from a desire to identify the genomic binding sites of heterochromatin protein 1 (HP1). DamID involves the fusion of Dam methylase to any protein (in this case HP1) and expression of the fusion construct in vivo. Wherever HP1 binds, the Dam methylase directs local DNA methylation. Then, DNA microarrays can be used to determine which DNA sequences are in vivo targets of HP1. This technique should be applicable to most organisms and is starting to be used in a number of laboratories.

The second story was the development of TILLING (Targeting Induced Local Lesions IN Genomes), a procedure in which point mutations in any gene can be isolated (McCallum et al., 2000; Colbert et al., 2001). The need for a new method to generate loss-of-function alleles in known genes grew out of graduate student Claire McCallum's frustrating attempts to generate mutants in the *CMT2* and *CMT3* genes. T-DNA knockouts were not found in the available collections, and attempts with antisense constructs also were unsuccessful, so McCallum developed the TILLING technique. DNA extracted from pools of M2 plants from ethyl methanesulfonate–mutagenized seeds are screened for the presence of point mutations in a gene of interest by polymerase chain reaction (PCR) amplification of the gene and analysis for single base pair mismatches. This can be done either by denaturing HPLC or, even more efficiently, by using the Cel1 enzyme (purified from celery), which cleaves DNA at mismatches. Henikoff gave an update on progress toward a National Science Foundation–sponsored service for the Arabidopsis community in which basic researchers could request TILLING alleles. He then explained several new algorithms that his laboratory has written to make the process of TILLING more efficient. TILLING has great potential because it can be applied to many organisms, and efforts are under way to generate TILLING populations in other species, such as maize.

FUNCTIONAL GENOMICS

Subcellular Protein Localization

Paul Dupree (Cambridge University, UK) presented work from his laboratory on the characterization of the protein complement of various plant organelles. Using the plasma membrane as an example, the Dupree group displayed plasma membrane proteins on high-resolution two-dimensional polyacrylamide gels and determined the identity of the most abundant proteins by mass spectroscopy. By comparing the complement of glycosyl phosphatidylinositol (GPI)-anchored proteins in all membranes with those in the plasma membrane or those secreted into the culture medium, they were able to demonstrate that most GPI-anchored proteins

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are located in the plasma membrane of callus tissue. These experiments suggested that GPI-anchored proteins are very abundant at the cell surface and that such proteins can be released from the plasma membrane by phospholipase C or D in a regulated fashion. GPI-anchored proteins have been implicated in cell-to-cell communication, transmembrane signal transduction, and the asymmetric distribution of membrane proteins in nonplant systems. Discovering the role for Arabidopsis GPI proteins should be very productive. In future work, the Dupree group will focus on the identification of the subcellular localization of novel proteins. They will offer proteomic analysis of Arabidopsis samples as a service via the Genomic Arabidopsis Resource Network (http://www.york.ac.uk/res/garnet/garnet.htm).

Metabolic Profiling

Arabidopsis is estimated to have more than 10,000 small metabolites, relatively few of which have been quantified systematically. Oliver Fiehn (Max Planck Institute for Molecular Plant Physiology, Potsdam, Germany) described the program that his group has developed to analyze small organic molecules in plant samples in a high-throughput manner using liquid and gas chromatography coupled to mass spectrometry. Metabolome analysis of individual Arabidopsis leaves suggested that each leaf has a unique identity, and experiments in which all leaves of a rosette are harvested together provide only an averaged view of leaf responses to a treatment. A number of metabolites were found to vary at statistically significant levels in the Col-0 and C24 compared with the F1 of these two accessions, suggesting that substantial metabolic diversity exists among natural accessions. In a powerful demonstration of the value of centralized databases, the coregulation of metabolites under a wide range of experimental conditions led Dr. Fiehn to propose the occurrence of new metabolic steps for the synthesis of citramalate, a compound not reported previously in plants, and to identify a candidate gene that might encode a citramalate biosynthetic enzyme. Such approaches will lead to the identification of new metabolites and to deeper insights into the integration of the various parts of plant metabolism.

Gene Deletion

Although profiling methods, such as transcriptome, proteome, and metabolome analysis, promise new insights into the functioning of Arabidopsis genes, they will not replace the need for mutants in reverse and forward genetic experiments. The use of T-DNA and transposable element insertional mutagenesis coupled with PCR-based screening is a well-established tool for reverse genetics in Arabidopsis. Dr. Yuelin Zhang (Maxygen-Davis, Davis, CA) described a mutant population enriched in deletion mutants and methods for PCR-based screening of this population for deletions of selected genes, called DELETEAGENE. A sizable number of gene family members occur as tandem duplicates in the Arabidopsis genome. If such genes have redundant or overlapping functions, it can be difficult to generate multiple mutant lines to assess gene function. The DELETEAGENE program is particularly well suited for this purpose. In a pilot project, deletions of 1 to 4 kb were typical, with 15.7 kb the largest deletion reported. Maxygen-Davis is proposing to provide access to this population as a service within the coming year.

Gene Silencing

Both conventional antisense and sense suppression approaches have been useful for targeted gene silencing, but they have been somewhat unpredictable and inefficient. Dr. Varsha Wesley (from Peter Waterhouse's group, Commonwealth Scientific and Industrial Research Organization–Plant Industry, Canberra, Australia) described how transgenes designed to express self-complementary (hairpin) RNA, containing sequences of the target gene, result in more reliable, efficient, and effective gene silencing. Using hairpin RNA constructs containing self-complementary regions ranging from 98 to 853 nucleotides resulted in efficient silencing in a wide range of plant species, and inclusion of an intron in these constructs had a consistently enhancing effect. Intron-containing hairpin RNA (ihpRNA) constructs generally gave ~90% of independent transgenic plants showing silencing. The degree of silencing with these constructs was much greater than that obtained using either sense or antisense suppression constructs. Wesley described a generic vector, pHANNIBAL, that allows a simple, single PCR product from a gene of interest to be converted easily into a highly effective ihpRNA silencing construct. She also described a high-throughput version of this vector, pHELLSGATE, which incorporates GATEWAY (Invitrogen, Carlsbad, CA) technology to replace the restriction enzyme digestion and ligation steps by a single in vitro recombination step. Like the DELETEAGENE strategy, gene silencing can be applied readily to a wide range of plant species for gene function analysis.

GENETIC MECHANISMS

Tetsuji Kakutani (National Institute of Genetics, Shizuoka, Japan) described the molecular characterization of the *clam* mutation, which is one of the developmental abnormalities that arose in the *decreased DNA methylation 1* (*ddm1*) mutant (Miura et al., 2001). *ddm1* mutants show an ~70% decrease in genomic methylation and display a host of defects, many of which can be segregated away from the original *ddm1*

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mutation and mapped to discrete loci. The *clam* phenotype was caused by the insertion of a new type of DNA transposon, called a CACTA element, into the *DWF4* locus. CACTA elements are similar to the En/Spm/Tam family of terminal inverted repeat transposons. Further analysis showed that wild-type Arabidopsis has a small family of CACTA elements that frequently do not transpose. But in *ddm1* mutants, the CACTA elements quickly multiply in the genome, transposing onto all five of the Arabidopsis chromosomes. These results confirm a long-standing hypothesis that a major function of DNA methylation is to control the activity of transposons.

Steve Jacobsen (University of California, Los Angeles) described a screen for genetic suppressors of a hypermethylated and silenced *SUPERMAN* allele called *clark kent* (Lindroth et al., 2001). This screen uncovered nine loss-of-function alleles of a new DNA methyltransferase called CHROMOMETHYLASE3. These *cmt3* mutants showed a nearly complete loss of DNA methylation at CpXpG sites, but almost no effect on CpG methylation, and displayed reactivated RNA expression of two retrotransposon sequences. These results show that CpXpG methylation is important for the maintenance of gene silencing at a subset of genes in plants and again suggest the importance of DNA methylation in transposon biology.

CELL BIOLOGY

A Temperature-Sensitive HEAT Repeat

The Arabidopsis genome is proving to be a useful conduit for identifying plant microtubule-associated proteins (MAPs), a process that has been frustratingly recalcitrant using conventional biochemical strategies. Geoffrey Wasteneys (Australian National University, Canberra) described the structure and function of a recently discovered MAP, MICROTUBULE ORGANIZATION 1 (MOR1). The Wasteneys laboratory identified MOR1 using an immunofluorescence-based screen for temperature-dependent microtubule disruption mutants (Whittington et al., 2001). Live imaging of microtubules using green fluorescent protein–tubulin constructs revealed how quickly microtubule arrays become short and misaligned at 29°C and how quickly (within minutes) they recover at 21°C. The two ethyl methanesulfonate–generated *mor1* mutant alleles have single amino acid substitutions in an N-terminal HEAT repeat, and Wasteneys discussed how this structural motif might function in cortical microtubule organization. MOR1 turns out to be a member of the XMAP215-TOGp family of MAPs, and these high-molecular-weight proteins share the N-terminal HEAT repeat identified in the Arabidopsis homolog. This is a good example of how subtle, conditional mutations can identify vital genes and also reveal functional motifs.

More Is Better

One puzzling feature of the *mor1* mutants is their lack of cell division phenotypes. Wasteneys speculated that lesions severe enough to affect MOR1's predicted role in organizing phragmoplast, preprophase band, and spindle microtubule arrays would be lethal. Corroborating this speculation, Dave Twell (University of Leicester, UK) announced in the next seminar that the homozygous-lethal *gemini pollen1* (*gem1*) mutant also is complemented by the Arabidopsis homolog of TOGp. The Twell laboratory identified the *gem1* and *gem2* mutants by screening for aberrant pollen cell division patterns (Park et al., 1998). In *gem1* and *gem2* mutants, some microspores show complex cell plate profiles and others divide symmetrically, causing both daughter cells to adopt vegetative cell fates, producing pollen with twin vegetative cells and no generative cell (Park and Twell, 2001). Reduced transmission of *gem1* gametes also has a female component, and Twell reported that cellularization of the embryo sac fails or is incomplete in *gem1*, revealing a further role for GEM1/MOR1 in gametophytic cytokinesis.

INDUCTIVE PROCESSES

Flowering

In the 1930s and later, M. Kh. Chailakhyan and others proposed that "flower-inducing hormone," or "florigen," is produced by leaves that have been subjected to favorable photoperiods and is transported to the shoot apex to induce flowering. Despite efforts for more than half a century and a recent revival of interest (Colasanti and Sundaresan, 2000), the molecular nature of florigen remains elusive. By contrast, we now know much about a central "floral repressor" encoded by the *FLOWERING LOCUS C* (*FLC*) gene for a MADS box transcription factor (Michaels and Amasino, 1999; Sheldon et al., 1999). *FLC* represses flowering by a rheostat-like mechanism, in which the level of *FLC* activity is proportional to the lateness in flowering. Two partially redundant and interacting pathways converge on the regulation of *FLC* expression. Through the vernalization pathway, prolonged exposure to cold reduces the levels of *FLC* transcript (Michaels and Amasino, 1999; Sheldon et al., 1999), and FLC protein levels parallel that of the transcript (Sheldon et al., 2000). Mutations in genes of another pathway (the autonomous pathway), such as *FCA* and *FVE*, cause the increase of *FLC* mRNA and protein levels (Michaels and Amasino, 1999; Sheldon et al., 1999, 2000), suggesting that the pathway also negatively regulates *FLC*.

A report by Jose Martínez-Zapater (Centro Nacional de Biotecnología, Madrid, Spain) on the molecular analysis of the *FVE* and *PRECOCIOUS* (*PRE*) genes provided a link between the

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autonomous and vernalization pathways and *FLC* regulation. *fve* mutants delay flowering by lengthening both the juvenile and adult vegetative phases in both short-day and long-day photoperiods. *FVE* encodes a WD-40 repeat protein known as AtMSI4, which belongs to a small protein family with five members in Arabidopsis. Members of this family of proteins are components of chromatin assembly factor-1, histone acetyltransferase, and histone deacetylase in eukaryotic organisms, and AtMSI1 was shown recently to be a component of Arabidopsis chromatin assembly factor-1 (Kaya et al., 2001). Thus, it is possible that the FVE/AtMSI4 protein is involved in chromatin remodeling and/or DNA methylation in the *FLC* locus via histone modifications. Interestingly, a homolog of *FVE*/*AtMSI4* from *Silene latifolia* has been reported as the first active gene identified in a plant Y chromosome (Delichère et al., 1999). By screening for suppressors of *fve*, Martínez-Zapater and colleagues identified a pleiotropic early-flowering mutant of a novel locus *PRE*. *pre* is epistatic not only to *fve* but also to another autonomous pathway mutation, *fca*. *PRE* encodes for a protein similar to human nucleoporin (NUP96) and may be involved in RNA or protein transport across the nuclear membrane.

FLC is a MADS box family transcription factor, and its transcriptional regulation is a key point in the regulation of flowering. Other important transcription factors include CONSTANS (CO) and AGAMOUS-LIKE 20 or SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 (AGL20/SOC1) (Samach et al., 2000). The Arabidopsis genome contains ~26,000 genes, of which 6% (~1550) are transcription factor genes (Riechmann et al., 2000). Naturally, the next question in the postgenome era is: how many transcription factors are involved in the regulation of flowering? Jose Riechmann and colleagues (Mendel Biotechnology, Hayward, CA) conducted a systematic survey of ~750 transcription factor genes. They found that in ~10% of cases, overexpression and/or loss of function affected flowering time, leading to an estimate that ~150 or so transcriptional factors are involved in the regulation of flowering. Riechmann and colleagues reported the detailed analysis of five *FLC* homologs, including *MADS AFFECTING FLOWERING 1* (*MAF1*) (Ratcliffe et al., 2001), which is also known as *AGAMOUS-LIKE 27* (*AGL27*) (Alvarez-Buylla et al., 2000) and *FLOWERING LOCUS M* (*FLM*) (Scortecci et al., 2001), underscoring the large degree of complexity involved in the regulation of flowering time. The genome approach surely will accomplish much more than filling in the gaps left by the conventional gene-by-gene approach.

Mechanisms Underlying Hormone Responses

Hormones are important regulators and coordinators of various inductive processes, including floral induction. The most notable progress in plant hormone research this year is that now we understand more about the perception of plant hormones (Inoue et al., 2001; Wang et al., 2001) and the interaction between hormone signaling and proteolysis (Schwechheimer et al., 2001). Because of the complexity of the downstream events, it is essential to understand the function of the hormone receptor.

Fernando Rodriguez (University of Wisconsin, Madison) spoke about the role of RAN1, a copper transporter, on the biogenesis of ethylene receptors. In a yeast mutant defective in copper transport, ethylene binding to ETR1 requires RAN1, consistent with the phenotype of the *ran1* mutants. Brassinosteroid (BR) is perceived by BRI1, a leucine-rich repeat (LRR)-containing receptor-like serine/threonine kinase. BRI1 was identified genetically by a unique screen that involved isolating BR-deficient or BR-insensitive mutants from a larger group of mutants exhibiting cabbage-like dwarfism (Li and Chory, 1997). All 18 BR-insensitive mutants fell into one complementation group, *bri1*. Kyoung Hee Nam (University of Michigan, Ann Arbor) adopted reverse genetics in combination with a two-hybrid screen and identified a novel BR-response molecule that was not identified by the previous "stringent" genetic screen. BRI1-interacting receptor-like kinase 1 (BIK1), one of the two-hybrid BRI1 kinase domain interactors, also encodes an LRR-containing receptor-like kinase. Is this BR receptor interactor also a specific component of BR signaling? Reverse genetics says yes. Loss of function of the *BIK1* gene causes mild cabbage-like morphology, whereas overexpression produces a phenotype reminiscent of BR overproduction. More importantly, BIK1 overexpression partially suppresses the *bri1* mutation, suggesting that BIK1 has overlapping function with BRI1.

Aux/IAA genes encode nuclear proteins with short half-lives that are involved in auxin-responsive element–mediated transcriptional regulation. The dominant mutations that stabilize these proteins, such as the *axr2* and *axr3* mutants, cause altered auxin response. Mark Estelle (University of Texas, Austin) reported that the turnover of the Aux/IAA protein is modulated by ubiquitin ligase SCF^{TIR1}. AXR2 interacts physically with SCF^{TIR1}, and its interaction is abolished by the *axr2-1* mutation. Moreover, the AXR2 protein is much more abundant in the *tir1-1* mutant, indicating that SCF^{TIR1} is involved in AXR2 degradation. Jan Smalle (University of Wisconsin, Madison) reported that T-DNA insertion into the RPN12a gene, which encodes a regulatory subunit of 26S proteasome, confers decreased cytokinin sensitivity in leaf expansion and root elongation growth, and altered expression of the cytokinin-regulated genes.

SEX AND REPRODUCTIVE PROCESSES

The reproduction session opened with an elegant genetic examination of female

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gametophyte development described by Norbert Huck (University of Zurich, Switzerland). Screens for segregation distortion and semisterile phenotypes (Moore et al., 1997) have identified mutants affecting all stages of megagametophyte development, from mitotic division and cellularization to fertilization and karyogamy. Strikingly, transposon mutagenesis has yielded a broader spectrum of mutants than has T-DNA mutagenesis (Christensen et al., 1998), most likely because T-DNA integration is thought to occur specifically at an early stage in the development of the haploid megagametophyte, so mutations with deleterious effects on later stages may be lost. The *feronia* mutation is late acting and gives morphologically normal gametophytes that do not, however, yield seed when fertilized. Microscopy indicates that although the pollen tube enters *feronia* gametophytes, it fails to burst and release its sperm cells. Because genetic analysis shows that *FERONIA* is only required maternally, it presumably affects signaling from the megagametophyte to the pollen tube. Further characterization of the *FERONIA* product should help elucidate more of the signaling components between the female and male gametophytes during fertilization.

Perhaps appropriately for a reproduction session, several talks centered on *AGAMOUS* (*AG*), a floral homeotic gene required to specify male and female reproductive organ fate. Jan Lohmann (Salk Institute, La Jolla, CA) discussed the activation of *AG* expression in the center of the floral meristem. Previous work had implicated *LEAFY* (*LFY*) as an activator, but because *LFY* is expressed throughout floral meristems, other spatially specific factors have been invoked. Compelling biochemical and genetic evidence was presented to show that WUSCHEL (WUS), which is expressed in the center of meristems, acts in combination with LFY to activate *AG* (Lenhard et al., 2001; Lohmann et al., 2001). Therefore, floral meristem patterning uses a component used more generally to pattern meristems. Another feature of *AG* is its role in limiting cell proliferation in the center of the floral meristem, which is determinate (unlike other meristems in Arabidopsis). *WUS* has an opposite role, to maintain stem cell populations, and it has been shown that *AG* acts to repress *WUS*, thereby creating a negative feedback loop (Lenhard et al., 2001; Lohmann et al., 2001). Further analysis of the interaction between the two genes should be rewarding. Anusak Pinyopich (University of California, San Diego) concluded the session by describing how the *SEEDSTICK* (*STK*) MADS box gene acts redundantly with the *SHATTERPROOF* MADS box genes to specify ovule identity. In *stk* single mutants, ovule development is relatively normal but seed abscission is prevented, a discovery with exciting agronomic implications.

METABOLISM AND METABOLIC REGULATION

Plants excel at synthesizing complex carbohydrates, from glycolipids to cell wall components to storage compounds. Glycolipids (i.e., the galactolipids and the sulfolipid sulfoquinovosyldiacylglycerol) are the main lipid constituents of photosynthetic membranes. Sulfolipid is remarkable because of its 6-deoxyglucose-6-sulfonate head group. Christoph Benning (Michigan State University, East Lansing) reported on the identification of two genes from Arabidopsis that are sufficient for the biosynthesis of sulfolipid when expressed in *Escherichia coli*. The first enzyme, SQD1, catalyzes the formation of the sulfolipid head group precursor UDP-sulfoquinovose from UDP-glucose and sulfite (Sanda et al., 2001). The second enzyme, SQD2, is presumed to be the glycosyltransferase required for sulfolipid assembly from UDP-sulfoquinovose and diacylglycerol. The respective sulfolipid-deficient *sqd2* knockout mutant should provide a definitive answer regarding the function of this lipid in seed plants.

Seed coat coloration is controlled by a complex regulatory network. Natalie Nesi (Institut National de la Recherche Agronomique Seed Laboratory, Versaille, France) reported that the *TRANSPARENT TESTA* genes *TT2* and *TT8* encode transcription factors that modulate the expression of seed coat flavonoid biosynthetic genes (Nesi et al., 2000, 2001). Of course, metabolism is not controlled only at the level of transcription. Hitoshi Onouchi (Hokkaido University, Japan) reported on the feedback regulation of mRNA stability of the cystathione-γ-synthase, an enzyme involved in methionine biosynthesis (Chiba et al., 1999). Apparently, *S*-adenosylmethionine is the effector for this feedback regulation.

PATTERN FORMATION AND CELL FATE DETERMINATION

Ben Scheres (University of Utrecht, The Netherlands) reported on the analysis of patterning mechanisms in the root meristem of Arabidopsis. Despite nearly constant lineage relationships, the Arabidopsis root displays astonishing developmental flexibility. Scheres' laboratory had shown previously that auxin is asymmetrically distributed in the root, with a peak concentration in the distal tip. Studies with *pin-formed* mutants that are defective in polar auxin transport, combined with laser ablation of single cells, had shown that the distribution of auxin controls cell fate and division patterns. In *hobbit* mutants, no proper root is formed and the root meristem appears to arrest at early stages of development. *HOBBIT* gene activity is required in the founder cells of the root meristem, and cloning of the gene revealed that the protein might be a component of the anaphase-promoting complex. Consistent with this, the gene is expressed

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throughout development in a cell cycle–dependent manner. Four new genes that control the development of the columella and lateral root cap were identified by promoter trapping and by screening for mutants that show altered expression of columella-specific marker genes, and initial studies suggest that they act downstream of auxin signaling.

Knotted-like homeobox genes play a central role in the establishment of the shoot meristem. A well-studied example in Arabidopsis is *STM* (*shoot meristemless*), which is required for the initiation and maintenance of the shoot meristem. One related gene, *KNAT1*, was identified many years ago, and overexpression of *KNAT1* in transgenic plants suggested that it might act in a manner similar to *STM* to promote meristem identity. However, loss-of-function mutants of *KNAT1* were not available. Scott Douglas (University of Toronto, Canada) surprised us with the identification of *brevipedicellus* (*bp*) as a *knat1* mutant (many have "studied" *bp* mutants before, because they decorate the Arabidopsis Biological Resource Center World Wide Web page). The *bp* phenotype is characterized by shortened internodes and pedicels, downward bends in pedicels, and turns at the nodes. *KNAT1*/*bp* had been shown to be expressed in the meristems and in pedicels, although the pedicel expression had attracted less attention. Interestingly, the *bp* phenotype is partially suppressed when the *ERECTA* gene, encoding an LRR receptor kinase, is functional. In *bp* pedicels, stripes of tissue that lack chloroplasts and intercellular spaces are found at the abaxial side, extending basipetally along the inflorescence stem. A similar reduction in chloroplast density is found adjacent to lateral organs of wild-type nodes. Douglas suggested that a vasculature-related repressor of chlorenchyma development acts at wild-type nodes and that both *KNAT1* and *ERECTA* are required to suppress its action in pedicels and internodes.

The shoot meristem remains active throughout plant development as a result of the presence of nondifferentiating stem cells in the central zone at the tip of the meristem. Rüdiger Simon (University of Cologne, Germany) summarized the regulation of stem cell fate by the homeobox gene *WUS* and the *CLAVATA* (*CLV*) signal transduction pathway. *WUS* is expressed in a deep region of the meristem, the organizing center, and promotes stem cell fate at the tip. *WUS* expression, in turn, is regulated negatively by the *CLV3* signal that is secreted from the stem cells. However, how these separate domains in the meristem are established is poorly understood. Rüdiger and Wolfgang Werr's laboratory (both in Cologne) used an activation tagging system based on the *Spm* transposon to identify *Dornröschen* (*Drn*; sleeping beauty), an *AP2*-related transcription factor that is expressed in stem cells and organ primordia. Increased expression of *Drn* in a gain-of-function mutant results in a drastic increase in meristem size, meristem arrest, and a rearrangement of the expression domains of *CLV3* and *WUS*. How *Drn* acts is not yet understood, and a loss-of-function allele is not (yet) available.

EVOLUTION

The study of natural variation in Arabidopsis is starting to receive a lot of attention. Combined with the wealth of genetic and developmental information available in this system, detailed studies of natural populations promise to enhance our understanding of evolutionary processes. Thomas Mitchell-Olds (Max Planck Institute of Chemical Ecology, Jena, Germany) talked about natural variation in Arabidopsis glucosinolate accumulation. Glucosinolates are secondary metabolites that influence plant–insect interactions, and most of their diversity is attributable to enzymatic modifications of a common structure (Kliebenstein et al., 2001). *MAM1* and *MAM2* are two loci implicated in glucosinolate elongation. Sequence analysis of these loci in a large number of individuals has revealed that polymorphism probably is maintained at least in the case of *MAM1* by balancing selection.

Michael Purugganan (North Carolina State University, Raleigh) is studying whether particular developmental genes are more prone to selection than others in natural Arabidopsis populations. He sequenced *AP3*, *PI*, *CAL*, *LFY*, and *TFL* in 19 Arabidopsis ecotypes and found that only *LFY* and *TFL* seem to have the significantly low level of variation that likely is the result of selective pressure. In the case of *TFL*, almost no variation is found in the coding region within ecotypes, and variation in the promoter region falls into two classes. These two classes correlate phenotypically with a small difference in the number of plant coflorescences.

Pilar Cubas (Universidad Autonoma de Madrid, Spain) presented research on the Arabidopsis *TCP* gene family. The *TCP* genes encode for proteins containing a predicted noncanonical basic helix-loop-helix domain thought to be involved in transcription regulation (Cubas et al., 1999). Members of this family such as *TEOSINTE BRANCHED-1* (*TB1*, from maize) and *CYCLOIDEA* (*CYC*, from Antirrhinum) have played key roles in the evolution of plant morphology. The 24 Arabidopsis *TCP* genes fall into two subfamilies, the *PCF* and the *CYC*/*TB1* subfamily. The putative orthologs of *TB1* (TCP12 and TCP18) and *CYC* (TCP1) form a small clade within the second subfamily. Their expression in axillary meristems may represent the ancestral expression pattern of *TB1* and *CYC* before they were recruited for new roles.

Jeffrey Chen (Texas A&M University, College Station) discussed the epigenetic control of gene expression found in Arabidopsis polyploids. Using amplified fragment length polymorphism-cDNA,

his group performed a genome-wide screen for orthologous genes silenced in allotetraploids derived from Arabidopsis and *Cardaminopsis arenosa*. They identified 10 genes that are silenced from either species. The silenced genes did not seem to be located in particular chromosomal regions, and they corresponded to a variety of RNA and proteins, including transcription factors (Lee and Chen, 2001).

RESPONSES TO THE ABIOTIC ENVIRONMENT

Photomorphogenesis

The COP1 E3 ubiquitin ligase plays a key role in photomorphogenesis. Predominantly cytoplasmic in the light, the COP1 protein accumulates in the nucleus in darkness, where it targets specific transcription factors, such as HY5, for degradation (Osterlund et al., 2000). To better characterize the role of COP1-interacting proteins in photomorphogenesis, Magnus Holm (Yale University, New Haven, CT) and colleagues isolated T-DNA insertion alleles for genes encoding HYH (Hy5 homolog) and CCO (COP1-interacting CONSTANS homolog), two proteins that were found to interact with COP1 in yeast two-hybrid experiments. Like *hy5* seedlings, *hyh* and *cco* seedlings flowered early and displayed long hypocotyls when exposed to blue light. The HY5 and HYH proteins were found to bind as homodimers or heterodimers to the G-box of light-responsive promoters. Furthermore, expression profiling of blue light–grown wild type, single mutants, and double mutants of *hy5* and *hyh*, and phenotypic analysis of *hy5 hyh* double mutants and of HYH-overexpressing seedlings, suggested that the two proteins cooperate in controlling the expression of light-regulated genes and in regulating chlorophyll accumulation.

The phytochrome family of photoreceptors also contributes to photomorphogenesis. Upon irradiation by red light, the Pr form of phytochrome is converted into its active Pfr form, which can activate the red light signaling pathway. Enamul Huq (University of California, Berkeley, and United States Department of Agriculture Plant Gene Expression Center, Albany, CA) reported on the identification and characterization of a basic helix-loop-helix protein, named PIF4, that appears to interact specifically with phyB in its Pfr form. *PIF4* overexpression resulted in red light hyposensitivity, whereas loss-of-function mutations at *PIF4* resulted in red light hypersensitivity. The data suggested that PIF4 functions as a negative regulator of phyB signaling, possibly by interacting with another bHLH protein, PIF3 (Martinez-Garcia et al., 2000), on the light-responsive G-box promoter element.

EARLY FLOWERING 3 (ELF3) is another phyB-interacting protein that may function as a transcriptional regulator (Hicks et al., 2001; Liu et al., 2001). Michael Covington (Scripps Research Institute, La Jolla, CA) discussed the involvement of *ELF3* in the regulation of circadian rhythms in Arabidopsis and proposed that the circadian regulation of *ELF3* expression enables the central oscillator to control the light sensitivity of both clock resetting and circadian outputs in a phase-specific manner (Covington et al., 2001).

Gravitropism

In roots, gravity perception by sedimentation of amyloplasts within the columella cells of the cap promotes a lateral redistribution of auxin. The resulting auxin gradient then is transported to the elongation zones, where it promotes curvature (reviewed by Chen et al., 1999). Auxin transport depends on the activity of an auxin efflux carrier complex containing a transmembrane protein encoded by members of the *AGR/EIR/PIN* gene family. Among these, AGR1/EIR1/PIN2/WAV6 contributes to the transport of auxin from the root cap to the elongation zones. Transport polarity appears to be associated with a polar distribution of this protein within the transporting cells. Rujin Chen (University of Wisconsin, Madison) showed that another member of this protein family, named AGR3, is localized at the periphery of columella cells, in a more or less symmetrical fashion, in vertically grown roots. However, when the roots were positioned horizontally, the AGR3 protein appeared to accumulate at the new physical bottom of the cells, at or near the plasma membrane, and the domain of AGR3 expression appeared to expand to peripheral cap cells at the bottom side. These data suggest that the gravity signal transduction pathway controls the trafficking of AGR protein(s) within the columella cells, creating a transport polarity that may contribute to the formation of an auxin gradient upon gravistimulation. It should be noted, however, that the regulation of auxin transport also might involve a reversible protein phosphorylation process, as discussed by Aaron Rashotte (Wake Forest University, Winston Salem, NC) (Rashotte et al., 2001).

In inflorescence stems, gravity is perceived by the sedimentation of amyloplasts within endodermal cells that surround the vasculature. Takehide Kato (Kyoto University, Japan) described two mutations, named *sgr2* and *sgr4/zig*, that affect shoot gravitropism. Both mutations appeared to affect vacuolar biogenesis and function in several tissues of the shoot, including the endodermis. Interestingly, amyloplasts often were pushed aside by large vacuoles in *sgr2* and *sgr4/zig* endodermal cells. *SGR2* encodes a novel phospholipase A1–like protein that localizes to the vacuole membrane, whereas *SGR4/ZIG* encodes a vacuolar SNARE protein (AtVTI1a) that may be involved in vesicle transport to the prevacuolar compartment. Hence, vacuolar biogenesis or function may play a role in gravity signaling in shoots.

MEETING REPORT

RESPONSES TO THE BIOTIC ENVIRONMENT

Research on pathogen recognition and the induction of plant defense responses is focused on the functions of *Resistance* (*R*) gene products in a number of laboratories. Andrew Bent (University of Wisconsin, Madison) gave a good introduction to this topic. *R* genes provide an efficient system for pathogen recognition in plants; recognition of a pathogen avirulence (*avr*) gene product by a plant *R* gene product triggers a plant defense system characterized by antimicrobial compound production, the hypersensitive response (a programmed cell death response at the site of infection), and subsequent induction of systemic acquired resistance. Most *R* genes examined to date encode LRR proteins, and many also carry a nucleotide binding site (NBS) domain. Bent presented biochemical evidence for nucleotide binding by the RPS2 protein and for disruption of defense function in plants when the *RPS2* gene carries mutations in the NBS.

The LRR domain now is recognized as a key factor in pathogen recognition. Work by Bent's group showed that in addition to its role in pathogen recognition, the LRR domain of the Arabidopsis *RPS2* gene also affects interactions with other factors (Banerjee et al., 2001). The Po-1 Arabidopsis accession is susceptible and the Col-0 accession is resistant to a *Pseudomonas syringae* carrying *avrRpt2*, and there are six amino acid differences between the RPS2 proteins from these accessions. Domain-swapping experiments showed that the LRR domain of RPS2 is a key factor for interactions with other host factors that influenced *RPS2*-mediated defense responses. Various studies have found that different NBS-LRR gene products use different signaling partners to activate defense responses (Warren et al., 1999; Feys and Parker, 2000). Bent also discussed microarray expression profiling work that further elucidates differences between different *R* gene signaling pathways.

David Mackey (from Jeff Dangl's group, University of North Carolina, Chapel Hill) presented evidence that the functioning of RPM1, another *R* gene product, requires RPM1-Interacting Protein 4 (RIN4). The Arabidopsis *RIN4* gene was identified in a yeast two-hybrid screen for proteins that interact with the *P. syringae* protein AvrB, and it also interacts with the N terminus of RPM1 in the yeast system. A *rin4* knockout plant line was used to show that RIN4 is required for RPM1-mediated inhibition of bacterial growth. The *rin4* knockout plants also exhibit constitutively increased pathogenesis related gene expression and other constitutive defenses. Work with plant extracts suggested that the RIN4 protein is localized to the plasma membrane and showed interaction between RIN4 and AvrB, AvrRpm1, and RPM1 proteins. Mackey discussed the "guard" hypothesis for RPM1 function, in which RIN4 is a negative regulator of defense whose association with AvrRpm1 in a susceptible host leads to repression of defense, but in a resistant host RPM1 either binds the RIN4/Avr-Rpm1 complex or prevents RIN4/Avr-Rpm1 binding, preventing repression of defense and instead causing strong defense activation (D. Mackey, B. Holt III, A. Wiig, and J.L. Dangl, unpublished results).

Paul Muskett (working with Jane Parker at the Sainsbury Laboratory, Norwich, UK) presented work on *Rar1*, a component of disease resistance signaling identified in a screen for the loss of *RPP5*-mediated resistance to *Peronospora parasitica*. RPP5 is another NBS-LRR *R* gene–encoded protein. The mutant identified in this screen was found to encode a homolog of the *Rar1* gene in barley, which was identified previously as being required for *Mla*-mediated resistance to barley powdery mildew (Shirasu et al., 1999). Muskett investigated the disease response using a variety of strains of *P. parasitica* and *P. syringae* and found that *Rar1* was required for some but not all *R* gene–mediated responses tested. Arabidopsis *rar1* mutants have been identified in four different laboratories that study plant disease resistance.

Mark Austin (also with Jane Parker at the Sainsbury Laboratory) provided genetic evidence for a link between Arabidopsis *SGT1* and *R* gene–mediated resistance to pathogens. SGT1 is an essential component of the SCF E3 ubiquitin ligase complex in yeast, and the Arabidopsis genome has two genes that encode SGT1-like proteins, named *SGT1a* and *SGT1b*. One mutant, *rpr1*, showing loss of *RPP5*-mediated resistance to *P. parasitica*, was found to have a mutation in the *AtSGT1b* gene. These data suggest a possible link between ubiquitin-targeted protein degradation and disease resistance.

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REFERENCES

Alvarez-Buylla, E.R., Liljegren, S.J., Pelaz, S., Gold, S.E., Burgeff, C., Ditta, G.S., Vergara-Silva, F., and Yanofsky, M.F. (2000). MADS-box gene evolution beyond flowers: Expression in pollen, endosperm, guard cells, roots and trichomes. Plant J. **24,** 457–466.

Banerjee, D., Zhang, Z., and Bent, A.F. (2001). The LRR domain can determine effective interaction between *RPS2* and other host factors in Arabidopsis *RPS2*-mediated disease resistance. Genetics **158,** 439–450.

Chen, R., Rosen, E., and Masson, P. (1999). Gravitropism in higher plants. Plant Physiol. **120,** 343–350.

Chiba, Y., Ishikawa, M., Kijima, F., Tyson, R.H., Kim, J., Yamamoto, A., Nambara, E., Leustek, T., Wallsgrove, R.M., and Naito, S. (1999). Evidence for autoregulation of cystathione gamma-synthase mRNA stability in Arabidopsis. Science **286,** 1371–1374.

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.

Colasanti, J., and Sundaresan, V. (2000). 'Florigen' enters the molecular age: Long-distance signals that cause plants to flower. Trends Biochem. Sci. **25,** 236–240.

Colbert, T., Till, B.J., Tompa, R., Reynolds, S., Steine, M.N., Yeung, A.T., McCallum, C.M., Comai, L., and Henikoff, S. (2001). High-throughput screening for induced point mutations. Plant Physiol. **126,** 480–484.

Covington, M., Panda, S., Liu, X., Strayer, C., Wagner, D., and Kay, S. (2001). ELF3 modulates resetting of the circadian clock in Arabidopsis. Plant Cell **13,** 1305–1315.

Cubas, P., Lauter, N., Doebley, J., and Coen, E. (1999). The TCP domain: A motif found in proteins regulating plant growth and development. Plant J. **18,** 215–222.

Delichère, C., Veuskens, J., Hernould, M., Barbacar, N., Mouras, A., Negrutiu, I., and Monéger, F. (1999). *SIY1*, the first active gene cloned from a plant Y chromosome, encodes a WD-repeat protein. EMBO J. **18,** 4169–4179.

Feys, B.J., and Parker, J.E. (2000). Interplay of signaling pathways in plant disease resistance. Trends Genet. **16,** 449–455.

Hicks, K., Albertson, T., and Wagner, D. (2001). *EARLY FLOWERING3* encodes a novel protein that regulates circadian clock function and flowering in Arabidopsis. Plant Cell **13,** 1281–1292.

Inoue, T., Higuchi, M., Hashimoto, Y., Seki, M., Kobayashi, M., Kato, T., Tabata, S., Shinozaki, K., and Kakimoto, T. (2001). Identification of CRE1 as a cytokinin receptor from Arabidopsis. Nature **409,** 1060–1063.

Kaya, H., Shibahara, K., Taoka, K., Iwabuchi, M., Stillman, B., and Araki, T. (2001). *FASCIATA* genes for chromatin assembly factor-1 in Arabidopsis maintain the cellular organization of apical meristems. Cell **104,** 131–142.

Kliebenstein, D.J., Kroymann, J., Brown, P., Figuth, A., Pedersen, D., Gershenzon, J., and Mitchell-Olds, T. (2001). Genetic control of natural variation in *Arabidopsis thaliana* glucosinolate accumulation. Plant Physiol. **126,** 811–825.

Lee, H.S., and Chen, Z.J. (2001). Protein-coding genes are epigenetically regulated in Arabidopsis polyploids. Proc. Natl. Acad. Sci. USA **98,** 6753–6758.

Lenhard, M., Bohnert, A., Jurgens, G., and Laux, T. (2001). Termination of stem cell maintenance in Arabidopsis floral meristems by interactions between WUSCHEL and AGAMOUS. Cell **105,** 805–814.

Li, J., and Chory, J. (1997). A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction. Cell **90,** 929–938.

Lindroth, A.M., Cao, X., Jackson, J.P., Zilberman, D., McCallum, C.M., Henikoff, S., and Jacobsen, S.E. (2001). Requirement of CHROMOMETHYLASE3 for maintenance of CpXpG methylation. Science **292,** 2077–2080.

Liu, X., Covington, M., Fankhauser, C., Chory, J., and Wagner, D. (2001). ELF3 encodes a circadian clock-regulated nuclear protein that functions in an Arabidopsis PHYB signal transduction pathway. Plant Cell **13,** 1293–1304.

Lohmann, J.U., Hong, R.L., Hobe, M., Busch, M.A., Parcy, F., Simon, R., and Weigel, D. (2001). A molecular link between stem cell regulation and floral patterning in Arabidopsis. Cell **105,** 793–803.

Martinez-Garcia, J., Huq, E., and Quail, P. (2000). Direct targeting of light signals to a promoter-element-bound transcription factor. Science **288,** 859–863.

McCallum, C.M., Comai, L., Greene, E.A., and Henikoff, S. (2000). Targeting Induced Local Lesions IN Genomes (TILLING) for plant functional genomics. Plant Physiol. **123,** 439–442.

Michaels, S.D., and Amasino, R.M. (1999). *FLOWERING LOCUS C* encodes a novel MADS-box domain protein that acts as a repressor of flowering. Plant Cell **11,** 949–956.

Miura, A., Yonebayashi, S., Watanabe, K., Toyama, T., Shimada, H., and Kakutani, T. (2001). Mobilization of transposons by a mutation abolishing full DNA methylation in Arabidopsis. Nature **411,** 212–214.

Moore, J.M., Calzada, J.P., Gagliano, W., and Grossniklaus, U. (1997). Genetic characterization of *hadad*, a mutant disrupting female gametogenesis in *Arabidopsis thaliana*. Cold Spring Harbor Symp. Quant. Biol. **62,** 35–47.

Nesi, N., Debeaujon, I., Jond, C., Pelletier, G., Caboche, M., and Lepiniec, L. (2000).

MEETING REPORT

The TT8 gene encodes a basic helix-loop-helix domain protein required for expression of DFR and BAN genes in Arabidopsis siliques. Plant Cell **12,** 1863–1878.

Nesi, N., Jond, C., Debeaujon, I., Caboche, M., and Lepiniec, L. (2001). The Arabidopsis *TT2* gene encodes an R2R3 MYB domain protein that acts as a key determinant for proanthocyanidin accumulation in developing seed. Plant Cell **13,** 2099–2114.

Osterlund, M., Wei, N., and Deng, X. (2000). The roles of photoreceptor systems and the COP1-targeted destabilization of HY5 in light control of Arabidopsis seedling development. Plant Physiol. **124,** 1520–1524.

Park, S.K., and Twell, D. (2001). Novel patterns of ectopic cell plate growth and lipid body distribution in the Arabidopsis gemini pollen1 mutant. Plant Physiol. **126,** 899–909.

Park, S.K., Howden, R., and Twell, D. (1998). The *Arabidopsis thaliana* gametophytic mutation gemini pollen1 disrupts microspore polarity, division asymmetry and pollen cell fate. Development **125,** 3789–3799.

Rashotte, A., DeLong, A., and Muday, G. (2001). Genetic and chemical reductions in protein phosphatase activity alter auxin transport, gravity response and lateral root growth. Plant Cell **13,** 1683–1696.

Ratcliffe, O.J., Nadzan, G.C., Reuber, T.L., and Riechmann, J.L. (2001). Regulation of flowering in Arabidopsis by an FLC homologue. Plant Physiol. **126,** 122–132.

Riechmann, J.L., et al. (2000). Arabidopsis transcription factors: Genome-wide comparative analysis among eukaryotes. Science **290,** 2105–2110.

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 of Arabidopsis. Science **288,** 1613–1616.

Sanda, S., Leustek, T., Theisen, M.J., Garavito, R.M., and Benning, C. (2001). Recombinant Arabidopsis SQD1 converts UDP-glucose and sulfite to the sulfolipid head group precursor UDP-sulfoquinovose in vitro. J. Biol. Chem. **276,** 3941–3946.

Schwechheimer, C., Serino, G., Callis, J., Crosby, W.L., Lyapina, S., Deshaies, R.J., Gray, W.M., Estelle, M., and Deng, X.W. (2001). Interactions of the COP9 signalosome with the E3 ubiquitin ligase SCF^{TIR1} in mediating auxin response. Science **292,** 1379–1382.

Scortecci, K.C., Michaels, S.D., and Amasino, R.M. (2001). Identification of a MADS-box gene, *FLOWERING LOCUS M*, that represses flowering. Plant J. **26,** 229–236.

Sheldon, C.C., Burn, J.E., Perez, P.P., Metzger, J., Edwards, J.A., Peacock, W.J., and Dennis, E.S. (1999). The *FLF* MADS box gene: A repressor of flowering in Arabidopsis regulated by vernalization and methylation. Plant Cell **11,** 445–458.

Sheldon, C.C., Rouse, D.T., Finnegan, J., Peacock, W.J., and Dennis, E.S. (2000). The molecular basis of vernalization: The central role of *FLOWERING LOCUS C* (*FLC*). Proc. Natl. Acad. Sci. USA **97,** 3753–3758.

Shirasu, K., Lahaye, T., Tan, M.W., Zhou, F., Azevedo, C., and Schulze-Lefert, P. (1999). A novel class of eukaryotic zinc-binding proteins is required for disease resistance signaling in barley and development in *C. elegans*. Cell **99,** 355–366.

van Steensel, B., Delrow, J., and Henikoff, S. (2001). Chromatin profiling using targeted DNA adenine methyltransferase. Nat. Genet. **27,** 304–308.

Wang, Z.Y., Seto, H., Fujioka, S., Yoshida, S., and Chory, J. (2001). BRI1 is a critical component of a plasma-membrane receptor for plant steroids. Nature **410,** 380–383.

Warren, R.F., Merritt, P.M., Holub, E., and Innes, R.W. (1999). Identification of three putative signal transduction genes involved in *R* gene-specified disease resistance in Arabidopsis. Genetics **152,** 401–412.

Whittington, A.T., Vugrek, O., Wei, K.J., Hasenbein, N.G., Sugimoto, K., Rashbrooke, M.C., and Wasteneys, G.O. (2001). MOR1 is essential for organizing cortical microtubules in plants. Nature **411,** 610–613.