MEETING REPORT

Arabidopsis Research Heats Up in Seville

Seville, Spain, was the setting of the 13th International Conference on Arabidopsis Research from June 28 to July 2, 2002. This fascinating city, with its spectacular gardens, beautiful Mudéjar royal palaces, and Gothic cathedral, provided an excellent backdrop for the conference, and the staff and facilities at the Seville Conference and Exhibition Center handled the 900-plus conferees with commendable smoothness and efficiency. Complete abstracts of the meeting can be found at http://www.arabidopsis2002.com/. We report here on some of our favorite presentations, with apologies to those whose work could not be cited as a result of space limitations.

THE ARABIDOPSIS FUNCTIONAL GENOMICS NETWORK

The Arabidopsis Functional Genomics Network is a 10-year program launched in November 2001 with support from the Deutsche Forschungsgemeinschaft. There are currently 11 contributing members and three associated groups. The coordinator of the network is Lutz Nover. The focus of the initiative is the functional analysis of multiprotein families, including analysis of all members of a given multiprotein family with respect to their expression and function during the Arabidopsis life cycle. Abstracts of the projects and additional information are available at http://www.uni-frankfurt.de/fb15/botanik/mcb/AFGN/AFGNHome.html.

Functional Analysis of MLO Proteins

Ralph Panstruga (working with Paul Schulze-Lefert at the Max Planck Institut, Cologne, Germany) reported on the functional analysis of the heptahelical transmembrane (7-TM) MLO protein family in Arabidopsis. Barley MLO has been identified as the prototype of a family of 7-TM proteins in plants (Büsches et al., 1997; Devoto et al., 1999). Lack of the wild-type protein leads to broad-spectrum disease resistance against the pathogenic powdery mildew fungus Blumeria graminis f. sp hordei. The Arabidopsis genome encodes 15 Mlo family members, and each of the genes is expressed. It has been shown that MLO mediates defense modulation via direct Ca\(^{2+}\)-dependent interaction with calmodulin and independently of heterotrimeric G-proteins (Kim et al., 2002). The current project aims to identify the functions of the 15 MLO family members in Arabidopsis. Systematic knockout of multiple Arabidopsis Mlo genes will allow the determination of the role of one or several family members in plant defense or other biological processes.

SCF Ubiquitin Ligase Complexes Related to EID1

Thomas Kretsch (University of Freiburg, Germany) presented a project aimed at determining the function of SCF ubiquitin ligase complexes. His group is particularly interested in determining the function of the F-box protein EID1, a component of phytochrome A-dependent light regulation in Arabidopsis (Dieterle et al., 2001). They want to examine the composition of SCF complexes related to EID1 and its homologs. Interestingly, EID1 shares similarity with proteins similar to the circadian clock coupling factor ZGT (Xu and Johnson, 2001). Domain-swapping experiments between EID1 and different F-box domains of other proteins involved in Arabidopsis signal transduction also will be performed. In vitro competition assays using matrix-bound ASK proteins and labeled F-box proteins will be used to elucidate the binding between these SCF complex components.

NATIONAL SCIENCE FOUNDATION 2010 PROJECT

The U.S. National Science Foundation 2010 Project is an ambitious funding program designed to determine the functions of 25,000 genes in Arabidopsis by the year 2010. More than $50 million was awarded in 2001 for 29 different projects for a period of 2 to 4 years each. A complete list of awardees and project abstracts can be found at http://www.nsf.gov/search97cgi/vtopic.

Arabidopsis Haplotype Map

Magnus Nordborg (University of Southern California, Los Angeles), in collaboration with Martin Kreitman and Joy Bergelson (University of Chicago, IL), will conduct a genomic survey of polymorphisms in Arabidopsis by sequencing ~2000 0.5- to 0.7-kb chromosomal segments spaced ~50 kb apart in 96 different accessions. The project will generate data that should aid in the identification of quantitative trait loci (QTLs) and in gene function studies as well as provide valuable information on species history and evolution. The data will be publicly available through GenBank as well as through a relational database being developed by the group (the project can be followed at http://magnolia.usc.edu/2010.html). Initial results from the sequencing of 36 fragments on chromosome 1 confirmed previous results (Nordborg et al., 2002) that linkage disequilibrium in
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Arabidopsis decays within 1 centimorgan (~250 kb) and showed clear evidence for population structure.

Seed Development

David Meinke (Oklahoma State University, Stillwater) is involved in a project to determine the essential gene functions in Arabidopsis seed development. The project is focused on nonredundant genes that produce an embryo-defective or seed pigment phenotype when disrupted. Meinke and colleagues have estimated that there are 750 to 1000 such genes in the genome (McElver et al., 2001). The project is a collaborative effort with researchers from Syngenta (Research Triangle Park, NC) and the Virginia Bioinformatics Institute (Blacksburg, VA). Syngenta has agreed to release gene identities and seed stocks with “no strings attached,” which Meinke cited as an important advance in making public resources available from investments made in the private sector. Details of the project can be found at http://www.seedgenes.org. Meinke presented a poster on a related project to produce a complete list of cloned Arabidopsis genes with a known mutant phenotype, and he is requesting help from the scientific community in completing the list. More information can be found through The Arabidopsis Information Service at http://www.arabidopsis.org.

P450 Genes

Mary Schuler (University of Illinois, Urbana-Champaign) described a project designed to define the expression patterns of all (several hundred) of the Arabidopsis cytochrome P450 monoxygenase genes and substrate reactivities of a subset of the P450 proteins. P450 proteins mediate oxidative reactions in a wide variety of biosynthetic and metabolic pathways, including lignin biosynthesis and defense-related phenylpropanoid metabolism. The project includes the construction of P450-specific microarrays for gene expression analysis and the development of an interactive World Wide Web site linked to The Arabidopsis Information Resource that will provide information on available ESTs, full-length sequences, expression patterns, etc.

Transcriptional Profiling of Plant Defense Responses

Xinnian Dong (Duke University, Durham, NC) presented a collaborative project with Frederick M. Ausubel (Massachusetts General Hospital, Boston, MA) and Shauna Somerville (Carnegie Institute, Stanford University, CA) that aims to use large-scale transcript profiling of Arabidopsis defense-related mutants to develop a comprehensive model for plant defense responses. The construction of a microarray of defense-related genes (pathoarrays) and Arabidopsis defense-related mutants would help to define the expression signatures that result from the activation of defense pathways. They will use mutants with enhanced resistance to pathogens or those with compromised resistance gene signaling pathways (e.g., salicylic acid– or jasmonate/ethylene–dependent signaling pathways). A World Wide Web–accessible plant-microbe interaction database (http://genetics.mgh.harvard.edu/ausubelweb/nsf2010/NSF_2010.html) will be developed within the next 4 years.

Functional Analysis of Ubiquitin Protein Ligase Families

Increasing evidence suggests that the ubiquitin/proteasome proteolytic pathway plays an integral role in plant development, responsiveness to hormones, light, Suc, and defense responses. Judy Callis (University of California, Davis) introduced a collaborative project (along with William L. Crosby, Xing-Wang Deng, Mark Estelle, and Richard Vierstra) aimed at analyzing the predicted 900 different E3 ubiquitin ligases encoded by the Arabidopsis genome, representing ~3.5% of the proteome. Vierstra and colleagues (University of Wisconsin, Madison) recently completed a phylogenetic analysis of the F-box proteins in Arabidopsis. A yeast two-hybrid screen is being exploited to identify interacting proteins, which include possible substrates. The collaborators will examine gene expression patterns and localization using DNA microarrays and green fluorescent protein–E3 fusions. Biochemical ubiquitin ligase activity will be determined for a number of known F-box proteins and putative RING E3s. Mass spectrometry will be used to profile ubiquitinated proteins. Information gained will be made available on the project World Wide Web page.

A Knockout for Every Gene

Michael Sussman (University of Wisconsin, Madison) provided an entertaining discourse on reverse genetics and an overview of a project designed to produce and distribute knockout mutants for every Arabidopsis gene. Sussman emphasized the importance of the reverse genetics approach to determine gene function. Forward genetics mutational screens will not identify many genes that have “backups” in the genome, and the generation of “gene family” (double, triple, etc.) knockout mutants will be critical to analyze the functions of these genes. Also, the likelihood of producing a knockout mutant for small genes (e.g., 0.5 kb) using conventional T-DNA– or transposon-based insertional mutagenesis is very low. Sussman described a project designed to create knockout mutants by crossing plants carrying Ds transposon insertions to plants that carry the Ac transposase gene and screening progeny to find lines in which the transposon has moved to knock out a nearby small or tandemly arrayed gene. This project uses a new technology called the maskless array synthesizer, a bench-
top machine invented at the University of Wisconsin that makes high-density oligonucleotide arrays with a micromirror device without expensive and time-consuming masks. This technology may offer the possibility of isolating knockout plants in any crop species, without the use of T-DNA. These projects are part of the Arabidopsis Knockout Facility at the University of Wisconsin (http://www.biotech.wisc.edu/Arabidopsis), which has been providing insertional mutants to the plant research community since 1999. To date, >500 researchers have used the facility, and 55 to 60% of screens have produced a knockout plant.

MORE HIGH-THROUGHPUT GENE SILENCING

Chris Helliwell (Commonwealth Scientific and Industrial Research Organization [CSIRO] Plant Industry, Canberra, Australia) described another large-scale reverse genetics project (a collaboration with Peter Waterhouse and colleagues at CSIRO) with potential for creating gene family knockouts using gene silencing. Waterhouse and colleagues (Smith et al., 2000) demonstrated the efficacy of gene silencing in plants using inverted-repeat transgene constructs that encode a hairpin RNA structure. The most important applications are likely to be silencing of multiple members of gene families, especially if the family members are closely related (and therefore likely functionally redundant), and also knocking out of specific gene functions in ecotypes or mutants that are not being used in large-scale gene knock-out projects. Helliwell and colleagues demonstrated that conserved sequences from the PhyC or PhyD genes also silenced PhyB, but nonconserved sequences did not. Furthermore, effective silencing of two different genes could be achieved by the introduction of a single construct containing sequences corresponding to both genes.

VERNALIZATION AND THE TRANSITION TO FLOWERING

Caroline Dean (John Innes Centre, Norwich, UK) and Candice Sheldon (CSIRO Plant Industry) presented recent work regarding how vernalization and the autonomous pathway regulate flowering time. Vernalized seeds have a “mitotic memory” of vernalization. Separation between the timing and the effect of vernalization, and the lack of transmission to progeny, suggest an epigenetic basis of this phenomenon. FLC encodes a repressor of flowering that is central to the vernalization response. Early-flowering Arabidopsis accessions exhibit low expression of FLC and show no response to vernalization, whereas late-flowering accessions have high expression of FLC and are late flowering in the absence of vernalization but become early flowering and have reduced FLC expression after vernalization.

Sheldon presented results from FLC::GUS fusion constructs that showed that different regions of FLC are required for the two components of the vernalization response: initial repression and maintenance of repression. Both promoter and intragenic regions are required for the initial repression, and sequences within intron I are likely to be important for maintenance. Furthermore, the gene adjacent to FLC also is repressed by vernalization, indicating that this repression affects a wider region, rather than just the FLC gene, providing support for the idea that a modification of chromatin structure occurs.

Dean elaborated on the activity of two other genes, VRN1 and VRN2, which are required for the stable repression of FLC after vernalization. VRN2 encodes a polycomb group protein (Gendall et al., 2001), which are proteins known to be involved in the stabilization of chromatin structure. The FLC molecular phenotype in vrn1 and vm2 mutants indicates that both VRN proteins act to maintain the repression of FLC after vernalization but not to establish the repressed state (Gendall et al., 2001; Levy et al., 2002). Further experiments are under way to determine if vernalization and the activity of VRN genes cause a change in chromatin structure at the FLC locus. Dean and colleagues also have shown that the RNA binding protein FCA interacts through its WW repeat with the WD-40 protein FY and that these two proteins may work in concert to promote flowering by repressing FLC.

Yoo-Sun Noh (University of Wisconsin, Madison) spoke about the molecular cloning and characterization of ef57, an early-flowering suppressor mutant of the late-flowering fri phenotype. The gene encodes a SWI2/SNF2-like chromatin remodeling factor that regulates FLC function. Noh and colleagues proposed that EF57 may act as an integrator of the pathways that regulate FLC-dependent floral transition.

Takashi Araki (Kyoto University, Japan) focused on downstream events in floral induction pathways and presented work on the FD gene, which acts specifically downstream of the floral inducer FT. The fd mutation suppresses the early-flowering phenotype of plants expressing 35S::FT but not that caused by the expression of 35S::LFY or 35S::SOC1. FD encodes a basic domain/Leu zipper (bZIP) transcription factor that is able to interact with FT in yeast, suggesting that this interaction is important for FT function.

Phil Wigge (Salk Institute, La Jolla, CA) seeks to understand why two very closely related proteins such as FT and TFL1 have antagonistic roles in the promotion of flowering. Wigge reported on the finding of a bZIP transcription factor that is very similar to FD and, like FD, is able to interact with both FT and TFL1. The group proposes that the bZIP protein may recruit FT or TFL1 to specific target promoters and that a third, yet unidentified factor would give transcriptional activation ability to the FT complex.

LIGHT SIGNALING AND CIRCADIAN REGULATION

In recent years, Xing-Wang Deng and colleagues (Yale University, New Haven, CT) have been unmasking molecular components
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of a central signaling pathway involved in
the light control of photomorphogenesis,
defined by many of the COP/DFF/FUS
loci. As summarized by Deng, COP1 rep-
resents the rate-limiting component of this
pathway, possibly acting as part of an E3
ubiquitin ligase that targets positive regu-
lators of photomorphogenesis, such as
HY5 and HYH, for degradation in the ab-
sence of light (Holm et al., 2002). In this
process, the COP9 signalosome may re-
place the lid of the 26S proteosome and
act in concert with COP1 to repress pho-
томorphogenesis in the dark. The involve-
ment of ubiquitination and the proteosome
in this pathway is supported further by the
finding that COP10 encodes an E2 variant
ubiquitin-conjugating enzyme (Suzuki et
al., 2002) that could work as a heterodimer
with a canonical E2 enzyme and that links
COP1 and the COP9 complex both func-
tionally and physically.

Virtudes Mira-Rodado (working with Klaus
Harter, University of Freiburg) reported on
the functional interaction of Arabidopsis
Response Regulator4 (ARR4) with the N
terminus of PHYB (Sweere et al., 2001).
The relevance of the interaction was demon-
strated in transgenic plants overexpress-
ing ARR4, which were more sensitive to
red light, and in plants lacking ARR4,
which were less sensitive to red light. It
was further shown that the interaction with
ARR4 led to the stabilization of the active
form of PHYB.

Karen Kaczorowsky (University of Cali-
ifornia, Berkeley) is involved in the search
for mutants hyposensitive to red light.
Among a battery of new alleles of known
genes, two alleles of a previously unidenti-
fied gene involved in red light signaling
were found. Mutant plants had longer hy-
pocotyls under red and far-red light.
Positional cloning revealed that the lesion af-
fected a pseudo-response regulator, APPR7,
which belongs to a small family of cir-
cadian clock-regulated pseudo-response
regulators that includes the clock gene
TOC1. Members of this family lack the Asp
residue involved in a phosphoryl char-
acteristic of response regulators. Interest-
ingly, Paloma Más (Scripps Research
Institute, La Jolla, CA) reported that plants
lacking TOC1 function show defects in
deetiolation under white, red, and far-red
light, whereas no phenotype was ob-
erved under blue light and in darkness.
This finding reveals a new and unexpected
role for TOC1 as an element acting down-
stream of PHYB and PHYA in the control
of morphogenic responses. These plants
also helped to define the role of TOC1 in
the molecular clock mechanism, revealing
that it is essential for clock function under
red light and in darkness, whereas other
clock components may compensate par-
tially for its loss under blue light.

The ZTL/ADO1 family of proteins may
act at the interface between light signaling
and the core of the clock. Jose Jarillo (In-
istituto Nacional de Investigaciones Agrar-
ias, Madrid, Spain) reported on the pheno-
typic analysis of plants deficient in each of
the three family members, showing en-
hanced red light sensitivity for hypocotyl
growth inhibition and later flowering time
compared with any of the single mutants.

How the clock defines the phase of
output genes, such as the evening-
phased gene CAT3, was addressed by
Rob McClung (Dartmouth College, Han-
over, NH). McClung’s group showed in vivo
that a change of 1 bp in the evening ele-
ment present in the CAT3 promoter to the related
CCA1 binding site associated with a morn-
ing phase is enough to switch the phase of
expression from dusk to dawn.

THE PLASTID PROTEOME

The plastid proteome contains up to 3500
proteins, and ~500 of these are mem-
brane proteins. Klaas J. van Wijk (Depart-
ment of Plant Biology, Cornell University,
Ithaca, NY) reported on his work in collab-
oration with Gunnar von Heijne (Depart-
ment of Biochemistry and Biophysics,
Stockholm University, Sweden) aimed at
defining Arabidopsis plastid subprote-
omes and characterizing a number of plas-
tid-localized processes. The group is using
two-dimensional gels, mass matrix-assisted
laser desorption ionization time-of-flight
mass spectrometry, and nanoelectrospray
tandem mass spectrometry on chloroplast
proteins obtained from different subcel-
lular fractions. They quantitatively sepa-
rated the hydrophilic thylakoid proteome into
three subproteomes: the soluble thylakoid
lumenal proteome, the peripheral thyla-
kokid proteome, and a tightly membrane-
anchored peripheral proteome (Peltier et
al., 2002). They also identified a chloro-
 plast-localized 350-kD Clp core protease
complex likely to be involved in subcellular
protein housekeeping and the regulation
of plastid gene expression (Peltier et al.,
2001). Knockout mutants in different Clp
genes are being used for proteomics stud-
ies aimed at determining the effect of Clp
gene disruption on Clp complex composi-
tion. The experimental proteome data will
be used to train neural network subcellular
protein localization predictors to screen
the Arabidopsis genome for the complete
plastid proteome.

BIOTIC AND ABIOTIC
STRESS RESPONSES

Role of NPR1 in Systemic
Acquired Resistance

Systemic acquired resistance (SAR) is a
secondary defense response that can be
induced after infection by an avirulent
pathogen. Xinnian Dong presented data
on the Arabidopsis NPR1 protein, an es-
sential signaling component of SAR. Mo-
lecular genetic characterization showed
that the nuclear localization of NPR1 and
phosphatase activity is required for PR1
induction. Using a dominant-negative 3’
fragment of the transcriptional activator
TGA2 and a chimera reporter system, they
found that NPR1 interacts in vivo with
TGA2 and regulates the binding affinity of
TGA2 to DNA (Fan and Dong, 2002).
Therefore, NPR1 regulation of SAR-related
gene expression may occur through inter-
action with transcription factors.
Salicylate (SA) and jasmonate (JA) play an important role in differentially regulating induced plant defenses against pathogens and insects (Kunkel and Brooks, 2002; Ton et al., 2002). In collaboration with Corné M.J. Pieterse (Phytopathology, Utrecht University, The Netherlands), Dong and colleagues showed that NPR1 is involved in cross-talk between SA- and JA-dependent pathways. JA-responsive expression of the PDF1.2 gene is abolished in wild-type plants treated with JA and SA. This inhibitory effect of SA on JA-mediated gene expression is abolished in the npr1 mutant. Using an inducible system to control the localization of NPR1, they demonstrated that NPR1 suppresses JA signaling through a novel function in the cytosol.

RAR1 Function

Ken Shirasu (Sainsbury Laboratory, John Innes Centre) reported on the mechanism of RAR1 function in plant response to pathogen attack. Previous studies identified RAR1 as a convergence point acting early in a signaling pathway engaged by multiple R genes in both barley and Arabidopsis. Sgt1-like proteins have been identified as RAR1-interacting proteins in yeast, and RAR1 interacts in vivo with SGT1 in barley and Arabidopsis (Azevedo et al., 2002). SGT1 is a highly conserved single-copy gene in most eukaryotes, and it is required for cell cycle progression at the G1/S and G2/M transitions via regulation of the kinetochore assembly and the SCF ubiquitin ligase complex in yeast. AtSGT1a and AtSGT1b have been shown to be functional orthologs of yeast Sgt1. Furthermore, gene silencing of barley SGT1 and knockout studies in Arabidopsis revealed its requirement in R gene–stimulated disease resistance. The pattern of interaction involving RAR1, SGT1, and the SCF complex provokes models for a link between disease resistance and the ubiquitination machinery in plants. RAR1/SGT1 complexes might be required for the degradation of polyubiquitinated negative regulators of disease resistance responses and/or for the activation, by monoubiquitination, of positive regulators, or they might have a function in stabilizing R-protein complexes.

QTLs Associated with Freezing Tolerance

The Cvi accession of Arabidopsis has lower tolerance to freezing injury than Landsberg erecta (Ler). Julio Salinas (Instituto Nacional de Investigacion y Tecnologia Agraria y Alimentaria, Madrid, Spain) described efforts to identify QTLs associated with freezing tolerance using recombinant inbred lines of Cvi and Ler. QTLs on chromosomes 4 and 5 accounted for 20 and 11%, respectively, of the variation in freezing tolerance between the two accessions. The QTL on chromosome 4 was found to align with three CBF genes, which encode transcription factors that bind to the DRE/CRT element present in the promoters of some cold- and drought-inducible genes, and DNA sequence polymorphisms were found in this region. Furthermore, expression of the CBF genes, and of genes induced by cold (e.g., COR47 and KIN1) was shown to be significantly lower in Cvi than in Ler. Complementation experiments are under way to confirm whether or not CBF genes are responsible for the phenotype.

New Salt and Osmotic Stress Mutants

Mike Hasegawa and Ray Bressan (Purdue University, Lafayette, IN) described projects to identify determinants of salt tolerance and osmotic stress tolerance, respectively, by screening for mutations that alter salt or osmotic stress sensitivity in T-DNA–tagged populations. Hasegawa described several mutants that have enhanced NaCl sensitivity, including npct1, a recessive T-DNA mutation that disrupts a gene encoding a Na⁺-dependent phosphate transporter–like protein. Another screen identified hkt1 mutations as suppressors of Na⁺ hyper-sensitivity and K⁺ deficiency in sos3-1 mutants, implicating the AHKT1 protein in Na⁺ and K⁺ acquisition (Rus et al., 2001). Bressan described the osm1 mutant, which is impaired in stomatal function and hypersensitive to salt and moisture deficit. OSM1 was found to encode a SNARE syntaxin, which is consistent with research implicating SNAREs in stomatal behavior. Another interesting mutant was hos15, which was isolated as a hyperresponding luminescent mutant in transgenic plants expressing luciferase under the control of the Rd29A promoter. The hos15 mutant is hyperluminescent for cold, abscisic acid, and NaCl induction of the Rd29A promoter, but it is hypersensitive only to cold treatment. Despite the phenotype, the mutant showed unaffected expression of several other cold-responsive genes, which may suggest another pathway that is important in stress responses.

EMBRYOGENESIS AND SEED DEVELOPMENT

Abed Chaudhury (CSIRO Plant Industry) discussed epigenetic processes during seed development. In fis mutants (comprising fis1/mea, fis2, and fis3/fie), the endosperm forms without fertilization, and after fertilization, the embryo is arrested and endosperm tissue overproliferates. FIS1/MEA encodes a polycistron protein similar to Drosophila Enhancer of Zeste, and FIS3/FIE encodes a protein similar to Drosophila Extra Sex Combs, a WD-40 group protein. FIS2 encodes a zinc finger protein that shows homology with Drosophila SU(Z)12 and to the Arabidopsis proteins EMF2 and VRN2. Expression of a FIS2:GUS fusion protein showed that expression starts in the two haploid central nuclei that form the central cell. Experimental crosses showed that fis2 and fis1 homozygous mutants could be rescued by hypomethylation in the pollen donor even without paternal FIS function (i.e., a fis1 or fis2 homozygous female mutant pollinated by pollen from homozygous fis1 or fis2 mutants hypomethylated by antisense inhibition of the DNA methyltransferase.
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Claudia Köhler (Universität Zürich, Switzerland) applied a genomic approach to identify target genes of MEA. She reported on the identification of the type 1 MADS box gene PHERES (PHE; initially called TOM1) as a potential target. It is strongly upregulated in both mea and fie mutants, and inhibition of PHE expression directed by the MEA promoter partially rescues the mea phenotype. These results indicate that the mea and fie phenotypes are attributable, at least in part, to high levels of expression of PHE.

Jiri Friml (University of Tübingen, Germany) presented recent work on the implication of auxin in early embryogenesis. The mp and bdl mutants lack some basal pattern elements as a result of a defect during specification of the hypophysis. MP and BDL encode ARF5 and IAA12 auxin response regulators, respectively. It is proposed that auxin releases MP from the inhibition caused by BDL and thus allows MP to modify gene expression, leading to root meristem specification in a non-cell-autonomous manner. This process also may involve PIN auxin transporter regulators, which are important to establish an auxin gradient during embryogenesis.

HORMONES AND SIGNAL TRANSDUCTION

Auxin, SCFTIR1, and Aux/IAA Proteins

Auxin response in Arabidopsis is dependent on the SCFTIR1 ubiquitin ligase (E3) complex (Gray et al., 1999). Auxin response factors (ARFs) and Aux/IAAs are both large families of transcription factors that regulate the expression of auxin-inducible genes. Stefan Kepinski, working with Ottoline Leyser (Department of Biology, University of York, UK), and in collaboration with Mark Estelle (Institute for Cellular and Molecular Biology, University of Texas, Austin), reported on an elegant analysis demonstrating that the degradation of the AUX/IAA proteins AXR2 and AXR3 is regulated by auxin via the SCFTIR1 complex (Gray et al., 2001). Importantly, auxin accelerates the degradation of Aux/IAAs by promoting their interaction with SCFTIR1. The authors are currently trying to understand how changes in Aux/IAA levels affect the transcription of auxin-regulated genes.

JA Signaling

Plants respond to many biotic and abiotic stresses locally and systemically through JA signaling molecules (Turner et al., 2002). Separate JA-dependent and independent wound signal transduction pathways exist in Arabidopsis (Leon et al., 1998). Jose J. Sanchez-Serrano’s group (Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas, Madrid, Spain) is assessing the role of JA in both local and systemic wound-induced gene activation. They showed that as well as inducing the JA-responsive genes JR2, PDF1.2, and VSP, wounding also induces the expression of JA-unresponsive genes such as WR3 and CK. COI1 encodes an F-box protein shown to be a key component of the JA-dependent signal transduction pathway (Xie et al., 1998). Recently it also was shown to form an SCFCO1 complex in vivo in Arabidopsis, possibly regulating ubiquitination and the degradation of repressors of JA responses (Devoto et al., 2002). Fabienne Cartieaux, working with Sanchez-Serrano, has started to characterize the expression of wound- and JA-inducible genes after treatment with MG132, a proteasome inhibitor. Lack of expression of JA-responsive genes in the JA-insensitive coi1-1 mutant or in the presence of MG132 in JA-treated wild-type Arabidopsis plants would support the function of COI1 suggested above.

Two-Component Systems in Cytokinin and Ethylene Signaling

Jen Sheen (Massachusetts General Hospital) presented a dissection of the cytokinin signal transduction pathway (Hwang and Sheen, 2001). Based on the previous identification of two His kinases as cytokinin receptors, Sheen and colleagues pursued a functional genomics approach to determine whether any of the putative two-component elements present in the Arabidopsis genome were involved in the cytokinin signaling cascade. They identified components and events in the cascade: His kinases (AHK) detecting the signal, which is transmitted to the nucleus by phosphotransmitters (AHP) that modulate the activity of response regulators (ARR), which modulate gene expression.

Continuing with two-component systems, Uta Sweere (University of Freiburg) proposed a second ethylene signaling pathway downstream of ETR1 that includes AHP2 and ARR2. AHP2 is proposed to act as a pivotal element in the integration of several signals.

Brassinosteroid Signaling

Yanhai Yin (Salk Institute) spoke about the recent cloning and characterization of the bes1 mutant, which suppresses the brassinosteroid-insensitive phenotype of bri1 (Yin et al., 2002). BES1 belongs to a plant-specific family of nuclear proteins and acts as a positive element in the pathway. Its role in brassinosteroid signaling was shown by the observation that BES1 is phosphorylated and destabilized by the negative element BIN2 in the absence of hormone. Thus, the basic backbone BR1-BIN2-BES1 of the pathway resembles the architecture of the Wnt pathway in animals.
CELLULOSE SYNTHESIS AND THE CORTICAL CYTOSKELETON

Plant cell walls are complex composites of polysaccharides, proteins, and phenolic compounds. Very little is known about the processes involved in the biosynthesis, transport, and assembly of cell wall polymers. Fourier transform infrared (FTIR) microspectroscopy can be used to distinguish mutants with altered cell walls from the wild type. Herman Höfte (Laboratoire de Biologie Cellulaire, Institut National de la Recherche Agronomique, Versailles, France) is using this technique to isolate mutants with defects in cellulose or pectin synthesis as well as mutants with alterations in the cortical cytoskeleton. By hierarchical clustering of FTIR data, his group was able to identify alleles of the same locus even in the absence of other observable phenotypic changes. Höfte described the isolation and characterization of KOBITO1, which encodes a novel plasma membrane protein necessary for normal cellulose synthesis during cell expansion (Pagant et al., 2002), and QUASIMODO1, which encodes a putative membrane-bound glycosyltransferase required for normal pectin synthesis and cell adhesion in Arabidopsis (Bouton et al., 2002).

NEW CELL EXPANSION MUTANTS

One of most striking features of plant growth and development is the precise regulation of cell volume increase. Keiko Sugimoto, working with Maureen McCann (Department of Cell and Developmental Biology, John Innes Centre), conducted a screen to search for Arabidopsis mutants with reduced hypocotyl elongation and identified two mutants, hypcotyl6 (hyp6) and hypcotyl7 (hyp7), that are defective in cell expansion in different tissues of mature plants. HYP7 encodes the nucleus-localizing protein RHL1 (Schneider et al., 1998). FTIR microspectroscopy indicated that the cell walls of the mutants have normal levels of cellulose, but they are altered in pectin composition and the alignment of microfibrils is disrupted from the wild-type transverse array. These observations suggest that cellulose alignment is not necessary for organ strength but that this and other cell wall changes may affect the elasticity of the organ.

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