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MEETING REPORT

Plant Biology 2001

The quadrennial joint annual meetings of the American Society of Plant Biologists and the Canadian Society of Plant Physiologists took place July 21 to 25 in Providence, Rhode Island. We report here on just a few of our favorite presentations. A complete listing of abstracts can be found at http://www.rycomusa.com/aspp/.

AWARDS

Jan A.D. Zeevaart (Michigan State University–Department of Energy Plant Research Laboratory, East Lansing, MI) was awarded the 2000 Steven Hales Prize for his work during the last four decades on plant hormones and the regulation of plant growth. The award honors the Reverend Stephen Hales for his pioneering work in plant biology published in his 1727 book *Vegetable Staticks*. Zeevaart's many contributions to plant biology include elucidation of the biosynthetic pathways of abscisic acid (ABA) and gibberellins (GA), the roles of ABA in stomatal closure and seed germination, and the role of GA in photoperiodism. Currently, his laboratory is interested in the environmental regulation of ABA and GA biosynthesis. ABA accumulation is induced very rapidly by drought. Although the mechanism of stress perception is not known in this case, likely targets of this signaling cascade include the genes that regulate the oxidative cleavage of a carotenoid precursor to yield ABA. Zeevaart's group has shown that this point of regulation is the limiting step in ABA biosynthesis (Qin and Zeevaart, 1999). His laboratory also is working to understand how long-daylength increases GA biosynthesis in long day Arabidopsis accessions and in spinach (Xu et al., 1997). They have demonstrated that the expression of 20-oxidase, a key GA biosynthetic enzyme, naturally increases in these plants upon induction by long-days. Overexpression of this gene in the long day rosette plant *Nicotiana sylvestris* promotes stem elongation under short-day conditions.

The Best Paper in Plant Cell 2000 prize was awarded to Henri Batoko from Ian Moore's group (University of Oxford, UK) for his work on vesicle trafficking in the secretory system (Batoko et al., 2000). Batoko and colleagues made use of an in vivo membrane trafficking assay based on confocal microscopy observations of the differential fluorescence of secreted and endoplasmic reticulum–retained green fluorescent protein (GFP). They found that a dominant negative mutant version of a Rab1 GTPase inhibited the export of a normally secreted GFP construct, causing it to be retained within the endoplasmic reticulum. They also made the striking observation that the mutant Rab protein inhibited vectorial Golgi movement. These observations suggested that Golgi movement and protein traffic along the secretory pathway are related and may be coupled directly.

The Best Paper in Plant Physiology 2000 prize was awarded to Thomas Girke (Dow AgroSciences, San Diego, CA) for his paper on microarray analysis of Arabidopsis seed (Girke et al., 2000). A complete understanding of plant gene function must include information on the tissues in which each gene is expressed as well as expression characteristics under different physiological conditions. To learn more about which Arabidopsis genes are expressed specifically in seed, White et al. (2000) sequenced 11,000 expressed sequence tags from a developing seed cDNA library. The unique clones selected from this set of expressed sequence tags became the basis of the microarrays discussed by Girke et al. (2000). In this paper, Girke and colleagues provide many fascinating insights concerning metabolic routes for the conversion of photosynthate into oil in developing Arabidopsis seed. Undoubtedly, many scientists will continue to mine the enormous amount of data provided in this article for years to come.

FUNCTIONAL GENOMICS

There is a growing number of resources in functional genomics available to plant researchers. Fritz Schomburg from Richard Amasino's laboratory (University of Wisconsin, Madison) gave an overview of the uses and limitations of activation-tagged populations. Activation tagging is an insertional mutagenesis approach in which a gene regulatory region (enhancer) is introduced randomly into a plant genome via *Agrobacterium tumefaciens*–mediated transformation (for review, see Weigel et al., 2000). The enhancer may dominantly activate and/or broaden the pattern of expression of a nearby endogenous gene. This technique allows for the generation of gain-of-function mutants, which may be particularly useful for the analysis of members of multigene families for which knockout mutations show no phenotype as a result of redundancy or overlapping function. Upon phenotypic identification of an activation-tagged mutant of interest, the region flanking the T-DNA may be cloned by plasmid rescue, adaptor-mediated polymerase chain reaction, or thermal asymmetric interlaced (TAIL)-polymerase chain reaction.

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Bradley Till from Steve Henikoff's laboratory (Fred Hutchinson Cancer Research Center, Seattle, WA) described a second tool for the identification of plant mutants, TILLING (targeting-induced local lesions in genomes). A graduate student in the laboratory, Claire McCallum, originally developed this technique (McCallum et al., 2000; Colbert et al., 2001). TILLING allows researchers to identify an allelic series of point mutations in a gene of interest from a population of ethyl methanesulfonate–mutagenized plants. Henikoff's group has participated in the development of a high throughput approach to TILLING and is part of a National Science Foundation–funded effort to offer it as a service to the Arabidopsis community (see http://sparky.fhcrc.org:1080/home.html).

GENE SILENCING

Gene silencing occurs at the transcriptional (TGS) or the post-transcriptional (PTGS) level when homologous nucleic acid (DNA or RNA) sequences are recognized. TGS is based on DNA methylation in promoter regions, and PTGS is accomplished through RNA silencing, which occurs via targeted degradation of RNA transcripts. These two gene silencing processes are not independent but are connected through double-stranded RNAs, which are formed by RNA-dependent RNA polymerase activities on single-stranded RNA templates, by RNA viral replication, or by the transcription of DNA inverted repeat sequences. Double-stranded RNAs are spliced by dicer RNase III into small interfering RNAs (siRNAs) of ~25 nucleotides. The siRNAs can either target a homologous DNA region in the nucleus for methylation, leading to TGS if RNAs containing promoter sequences are involved, or induce the degradation of homologous mRNAs in the cytoplasm (PTGS) (Matzke et al., 2001).

Marjori Matzke (Austrian Academy of Sciences, Salzburg, Austria) described experiments that showed double-stranded RNA–induced methylation of the homologous promoter regions. When a silencer plant (hygromycin resistant) containing a 35S promoter–driven inverted repeat of nopaline synthase (NOS) promoter regions was crossed to a target plant containing the NOS promoter–driven *npt II* gene, kanamycin-sensitive plants occurred through methylation of the NOS promoter in the target plant. Matzke also presented an experiment suggesting that TGS functions as a defense mechanism against the invasion of DNA viruses. Tobacco endogenous pararetroviruses are endogenous multicopy viruses (500 to 1000 copies) in tobacco. The tobacco endogenous pararetroviruses promoter–driven β-glucuronidase reporter gene was not active in the tobacco plant as a result of methylation of the promoter but was active and unmethylated in Arabidopsis, in which the endogenous virus sequences were absent.

Small interfering RNAs occur at multiple sizes between 21 and 25 nucleotides. David Baulcombe (The Sainsbury Laboratory, Norwich, UK) and Vicki Vance (University of South Carolina, Columbia) reported that HC-Pro (helper component proteinase), a plant viral suppressor of PTGS, differentially suppresses the accumulation of siRNAs of various sizes, suggesting that alternate pathways produce the small RNAs. Baulcombe gave an update on the large scale VIGS (Virus-Induced Gene Silencing) screening project for forward and reverse epigenetics. The project has catalogued up to 5000 genes, and has led to the identification of some genes needed for disease resistance, such as *SGT1* (an SCF-type E3 ligase component) and *NRG1* (N requirement gene 1), a nucleotide binding site-leucine rich repeat protein.

Vicki Chandler (University of Arizona, Tucson) described work her group has conducted to determine mechanisms of paramutation, a form of TGS in which an allele can "convert" another allele to a stably silenced state. Using maize anthocyanin genes that are capable of paramutation, Chandler's group is searching for the molecular basis of epigenetic gene silencing control. Genetic screens for mutants affected in the paramutation process have identified several necessary genes, such as *MOP1* (*Mediator Of Paramutation 1*) (Dorweiler et al., 2000), which are thought to be involved in chromatin remodeling. Because the *mop1* mutant also is affected in transgene silencing, there appear to be common molecular mechanisms underpinning different types of silencing events. Mapping of recombination breakpoints has allowed Chandler's group to identify *cis*-acting elements required for paramutation. A 6-kb element that is 95 kb upstream from the paramutagenic allele *B′* is necessary for gene silencing, and this enhancer seems to work by affecting chromatin structure in the *B′* gene.

PATHOGEN DEFENSE

Pathogen defense responses are initiated upon pathogen recognition. The best understood pathogen systems involve the recognition of a specific avirulence (avr) gene product from the pathogen by a resistance (R) gene product of the plant in what is called a typical gene-for-gene interaction. Work by Gregory Martin's group (Boyce Thompson Institute, Ithaca, NY) has revealed that in some cases it may be more appropriate to talk about a genes-for-genes relationship. They have identified a homolog of the *Pseudomonas syringae* pv *tomato* avr gene AvrPto, named AvrPto2, that can interact with the same R gene in tomato, Pto kinase, to elicit a hypersensitive response. Studies in the tomato relative *Lycopersicon pimpinellifolium* suggest that

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there also may be multiple R genes that recognize AvrPto2.

Our increased understanding of avr-R gene relationships often has led to the "pyramiding" of R genes (the use of a combination of R genes in the same plant to combat pathogen infection), although this strategy has had only limited effectiveness in the presence of diverse pathogen populations. Dirk Hays (Kansas State University, Manhattan) discussed an exciting alternate method being attempted to increase the resistance of wheat to infection by wheat leaf rust. Wheat will be transformed with a transgene consisting of a promoter of a gene induced by virulent infection fused to the coding region of an avr protein. Because the plant stock used will contain the corresponding *R gene*, it is hoped that infection-induced expression of the avr protein in the plant will stimulate resistance via a signaling cascade initiated by the interaction between the avr protein and the *R gene*. This method may be a useful alternative to *R gene* pyramiding, because the plant would be able to maintain resistance regardless of the avr gene population of the pathogen and the *R gene* composition of the plant.

Andrew Bent's laboratory (University of Wisconsin) also is interested in how avr–*R gene* relationships allow for pathogen recognition and elicitation of downstream events. Using microarray analysis, this group is examining the Arabidopsis genes induced by various avr–*R gene* interactions. Interestingly, even for the same pathogen and host, different avr–*R gene* pairs result in overlapping but different sets of elicited genes, suggesting that the signaling pathways downstream of different *R genes* and the type of resistance encountered in each instance may not always be identical. Bent also discussed data-handling issues related to microarray studies and demonstrated, among other things, the important need for replication of chip experiments.

The production of a number of metabolites, including salicylic acid (SA), jasmonic acid (JA), and ethylene, increases after pathogen recognition, eliciting downstream effects. Several researchers, including Daniel Klessig (Boyce Thompson Institute) and Xinnian Dong (Duke University, Durham, NC), reported on suppressors of the SA-insensitive Arabidopsis mutant *npr1*, suggesting that systemic acquired resistance is both positively and negatively regulated in vivo. Klessig's group has cloned the suppressor *SSI2*, which has been shown to be a stearoyl–acyl carrier protein desaturase, converting steric acid (18:0) to oleic acid (18:1). Although SA signaling is induced in these plants, JA responses are impaired without affecting overall JA levels. Thus, SSI2 suppresses NPR1 responses and accentuates JA responses. This suggests that fatty acids or their metabolites may regulate the communication between SA and JA defense responses in plants (Kachroo et al., 2001).

In addition to the identification of the NPR1 negative regulator SNI1 (Li et al., 1999), Dong's group has focused on the mechanism of activation of NPR1. NPR1 has three putative nuclear localization sequences but lacks a DNA binding domain. Using NPR1-GFP and NPR1-glucocorticoid receptor domain fusions, they have shown that NPR1 is localized in the nucleus following treatment with the SA analog 2,6-dichloroisonicotinic acid. Nuclear localization of NPR1 is necessary but not sufficient to induce pathogenesis-related gene expression (Kinkema et al., 2000). Data suggest that NPR1 may be linked to the transcription of pathogenesis-related genes through interaction with members of the TGA subclass of b-ZIP transcription factors (Zhang et al., 1999).

Ethylene production also increases after infection. Previous studies have demonstrated that ethylene enhances cell death after infection with virulent pathogens, suggesting that it may be central to the hypersensitive response. Harry Klee's group (University of Florida, Gainesville) has shown that tomato plants with reduced expression of ethylene receptor genes have increased cell death, pathogenesis-related gene expression, and ethylene production upon exposure to an avirulent pathogen (Ciardi et al., 2001). These results suggest that the induction of ethylene receptor gene expression may act to downregulate the hypersensitive response and other defense responses to avirulent pathogens.

INSECT HERBIVORY

After years of studying molecular mechanisms of plant resistance to bacterial pathogens, Fred Ausubel (Massachusetts General Hospital, Boston) and his colleagues, including Naomi Pierce (Harvard University, Cambridge, MA), have begun a new project to define the mechanisms of plant resistance to insect herbivores. The group is using *Trichoplusia ni*, commonly known as cabbage looper, as a model insect herbivore of Arabidopsis. The larvae of this moth feed on Brassica crops as well as a wide variety of other species and readily consume Arabidopsis. However, there is a range of resistance to feeding by *T. ni* across Arabidopsis accessions. Ausubel's group chose two accessions, Landsberg *erecta* (L*er*) and Columbia (Col-0), to identify a locus involved in insect resistance. Whereas *T. ni* will consume shoots of L*er* plants nearly to completion, Col-0 plants will be only lightly grazed. Genetic mapping studies identified a region near 85 centimorgan on chromosome 1, named the *TASTY* locus, that corresponds to the susceptibility of L*er* × Col-0–derived recombinant inbred lines to consumption by *T. ni* (Jander et al., 2001). The locus is distinct from previously identified loci imparting herbivore resistance traits such as glucosinolate content, trichome density, and disease resistance. Interestingly, three-way studies involving plant infection with virulent or avirulent

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bacterial pathogens followed by exposure to *T. ni* indicate that there may be interactions between bacterial and insect defense pathways. Plants that successfully mounted a hypersensitive response to bacterial infection suffered less feeding damage after subsequent exposure to *T. ni*.

ENGINEERING SALT TOLERANCE

Eduardo Blumwald (University of California, Davis) presented his research on engineering salt tolerance in crops. This work is the result of many years of research on vacuolar Na/H antiports in plant cells. Na is toxic at a certain concentration within the cell. There are two ways for the cell to diminish Na concentration in the cytosol: the use of the pH gradient across the vacuolar membrane to exchange protons and accumulate Na through the operation of vacuolar Na/H antiporters, and the use of energy in plasma membrane antiporters to extrude Na out of the cells. Arabidopsis has a family of five vacuolar Na/H antiporters that show various expression levels and distributions within the plant. Overexpression of one of these genes, *AtNHX1*, resulted in plants that could grow in high concentrations—up to 200 mM—of NaCl. Overexpression of the Arabidopsis gene then was tested in a number of other species with similar results. The group reasoned that the use of this technology would be best suited to fruit crops, because the Na in overexpressors of *AtNHX1* accumulated specifically in leaf vacuoles. Indeed, overexpression of *AtNHX1* in tomato produced highly salt-tolerant plants that accumulated salt in the foliage but not in the fruit. These results could prove to be quite beneficial for agriculture, because a large and growing portion of the soils in the western United States and Florida have relatively high salt concentrations.

Roberto Gaxiola (University of Connecticut, Storrs) presented research showing that overexpression of the AVP1 vacuolar H^+-pyrophosphatase in Arabidopsis increases salt and drought tolerance. Surprisingly, plant size also was enhanced greatly because of an increase in cell number that resulted in an approximate doubling of the number of rosette leaves produced. Gaxiola hypothesized that the increased AVP1 activity, secondary to increased AVP1 protein in vacuolar membranes, facilitates biosynthetic reactions by scavenging the pyrophosphate from the cytosol and using it as a source of energy for active transport of H^+ into the expanding vacuoles of new cells (Gaxiola et al., 2001).

METABOLIC SIGNALING AND GENE REGULATION

Sugars are central to the regulation of many processes in plants. Past work in Jen Sheen's laboratory (Massachusetts General Hospital, Boston) has shown that glucose regulation of transcription is mediated in part through hexokinase, which acts as a sugar sensor. Sheen presented further evidence suggesting hexokinase's separate metabolic and signaling functions by demonstrating that there is no correlation between glucose phosphorylation and signaling activities in the *gin2* hexokinase mutant. Published data from that group have unraveled interactions between sugar signaling and ethylene (Zhou et al., 1998) and ABA (Arenas-Huertero et al., 2000) signaling pathways. Sheen now reveals that there also is an important positive relationship between sugar and auxin signaling and a negative relationship between sugar and cytokinin signaling, further demonstrating the centrality of sugar signaling in plants. The laboratory has used Affymetrix gene chips to discover new interactive pathways by comparing global gene expression patterns in wild-type and *gin2* plants.

Nigel Halford (University of Bristol, UK) focused his presentation on sucrose nonfermenting-1 (SNF1)–related protein kinase 1 (SnRK1), an intracellular modulator of plant sugar metabolism. SNF1, the yeast serine/threonine kinase (Celenza and Carlson, 1986), plays a central role in carbon partitioning through derepression of all glucose-repressed genes, phosphorylation of enzymes, and interactions with other signaling pathways. A smaller plant homolog, SnRK1, which is functionally exchangeable with SNF1 in yeast, has a similar role in allocating sugars to their different usage pathways. It has been shown that some key enzymes for sugar metabolism, such as 3-hydroxy-3-methyl glutaryl–CoA reductase (the enzyme needed for isoprenoid synthesis), nitrate reductase, and sucrose phosphate synthase, are regulated by SnRK1. Antisense experiments demonstrated the pivotal functions of SnRK1 during plant development in concert with sugar metabolism. Antisense inhibition of SnRK1 was found to inhibit bud sprouting and enhance leaf starch content in potato. In barley, antisense SnRK1 arrested pollen development at the binucleate stage. The alpha-amylase promoter was silenced by antisense SnRK1 in a wheat embryo transient expression system using promoter-gus fusions. Efforts to search for SnRK1-interacting proteins by the yeast two-hybrid system raised SnIT1, a transcription factor with an apetala-2 type DNA-binding domain, and SnIP1, a protein with low homology to SNF4 (the SNF1-interacting protein in yeast), and containing a motif suggestive of a pseudosubstrate site similar to those observed in the regulatory subunits of the cAMP-dependent kinase, PKA, of mammals (Taylor et al., 1990).

Amino acids, sugars, fatty acids, and other metabolic compounds also can act as signaling molecules in plants. Gloria Coruzzi's laboratory (New York University, New York) is examining how carbon, nitrogen, and light signals are detected and interact to reg-

ulate nitrogen assimilation. Coruzzi is using a systems approach to understand the effect of the combined interaction of these factors on the transcriptional regulation of genes in the amino acid biosynthetic pathway, including asparagine synthetase (ASN1), which converts Gln to Asn, an amino acid used for nitrogen transport and storage in the plant. Using a positive selection scheme involving a transgenic construct fusing the ASN1 promoter to the coding regions of selectable markers, the laboratory has identified *carbon repression insensitive* (*cri*) Arabidopsis mutants that are unable to repress ASN1 transcription in the presence of high sugar. The laboratory also is examining the roles of plant homologs of allosteric effectors that regulate glutamine synthetase in response to nitrogen availability in bacteria (Hsieh et al., 1998) and plant homologs of glutamate receptors (Chiu et al., 1999) in amino acid–sensing in plants.

BRASSINOSTEROID SIGNALING

Joanne Chory (The Salk Institute, La Jolla, CA) described recent progress in brassinosteroid (BR) signaling. BRs are steroid hormones that are involved in light-mediated patterns of development, cell expansion, vascular differentiation, senescence, reproductive development, and stress protection. Chory's group and others originally recognized the importance of BRs during plant development and identified genes involved in BR biosynthesis and signal transduction. Recently, Chory's group demonstrated that the putative BR receptor kinase BRI1 functions as a receptor of brassinolide (Wang et al., 2001b), and they are beginning to study related Arabidopsis genes. Other genes involved in BR signal transduction have been identified in genetic screens. Subsequent work with the *BRI* genes and mutants has indicated that there are interactions between the ABA and BR signal transduction pathways, a hypothesis that is being tested using ABA mutants.

JASMONATE SIGNALING AND DEFENSE RESPONSES

Edward Farmer (University of Lausanne, Switzerland) presented exciting new results on the role of oxygenated fatty acids (oxylipins) in wounding and defense responses. These compounds include the jasmonate family of molecules. Examining the resistance of various jasmonate pathway Arabidopsis mutants to the insect Bradysia revealed that whereas JA can play an important role in wounding and defense responses, these responses are not always dependent on JA itself. A jasmonate family member is required, however. Farmer hypothesized that JA can be replaced by a cyclopentenone precursor of JA. Because many jasmonate family members accumulate in wounded Arabidopsis plants, his group tried to determine if a small, isolated component common to some of these molecules could act as a defense signal (Vollenweider et al., 2000). They found that the small molecule methyl vinyl ketone could act in this manner but that the control molecule butan-2-one, which lacks a crucial double bond, could not. This minimal structure is found in various jasmonates, including the JA precursor oxo-phytodienoic acid, but not in JA, further suggesting the importance of cyclopentenone jasmonate family members in defense responses.

COORDINATION OF LIGHT SIGNALS

Xing-Wang Deng (Yale University, New Haven, CT) discussed the idea that light serves as a key signal for the coordination of many different pathways in the cell. Deng's group conducted gene expression analysis using microarrays containing 9216 Arabidopsis expressed sequence tag clones that correspond to ~6000 genes. They found that the same set, corresponding to approximately one third of the genes represented, showed strikingly similar expression profiles under many different types of light regimens. They concluded that the role of distinct photoreceptors is largely to turn on and off a common set of genes that is mediated by regulating the developmental switch defined by the COP/DET/FUS group of regulators, including COP1 and the COP9 signalosome. The COP group regulators promote the degradation of the transcription factors responsible for photomorphogenesis. Among them, COP1, with the help of the COP9 signalosome, acts as an E3 ubiquitin ligase to target certain proteins involved in photomorphogenesis, such as the HY5 transcription factor, for degradation. The E3 ligase SCF^{TIR1} mediates the response to auxin in Arabidopsis. Deng and colleagues have shown that COP9 signalosome partial loss-of-function mutants display an auxin-resistant phenotype and that the SCF^{TIR1} protein interacts with the COP9 signalosome in yeast two-hybrid assays (Schwechheimer et al., 2001). Thus, the COP9 signalosome appears to be a regulator of multiple E3s, which are key regulators of various developmental pathways.

CHLOROPLAST STROMULES

Maureen Hanson (Cornell University, Ithaca, NY) described observations from experiments in which plastids were visualized by expression of a nuclear transgene encoding plastid stroma-targeted GFP. In 1997, her laboratory reported that GFP localized to the stroma

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of chloroplasts allowed the visualization of unexpected structures: tubules that emanate from the chloroplast surface (Kohler et al., 1997). These structures were named "stromules." Similar structures had been observed in phase-contrast images of spinach leaf chloroplasts in 1962, but the fragility of the stromules prevents them from being visible during electron microscopy studies. The Cornell group conducted irradiation experiments with GFP plants and demonstrated that GFP can move from one plastid to another through the stromules. Their physiological function is unknown, but they are abundant in nonphotosynthetic tissues, such as petals and roots. Fluorescence correlation spectroscopy studies indicated that movement of GFP through these structures involves both passive and active components (Kohler et al., 2000).

NONPHOTOCHEMICAL QUENCHING AND PHOTOPROTECTION

Nonphotochemical quenching (NPQ) of chlorophyll fluorescence occurs when absorption of light energy exceeds a plant's capacity for photosynthetic carbon fixation, and it is thought to protect the photosynthetic apparatus from the otherwise photoinhibitory effects of excess light energy. Xiao-Ping Li (University of California, Berkeley) spoke about the isolation of Arabidopsis *npq* mutants by video imaging of chlorophyll fluorescence. Characterization of a number of *npq* mutants suggested that three elements are necessary for NPQ: reversible conversion of violaxanthin to zeaxanthin, maintenance of a pH gradient across the thylakoid membrane, and the presence of the PsbS protein. Li focused attention on *npq4* mutants, which have normal pigment composition and electron transport capacity but lack the PsbS protein and do not show rapidly reversible NPQ. PsbS is a 22-kD intrinsic protein of the photosystem II complex. It is encoded by a single gene in all green plants that have been studied, and it appears to be absolutely necessary for the maintenance of rapidly reversible NPQ. Interestingly, Li and colleagues found that the capacity for NPQ was dependent on the amount of PsbS protein. Overexpression of a second copy of *PsbS* in Arabidopsis, which resulted in a severalfold increase in the amount of PsbS protein in vivo, had the unexpected effect of doubling the capacity for NPQ and enhancing resistance to photoinhibition.

INTERCELLULAR SIGNALING

Intercellular signaling in many cases involves the activity of receptor-like kinases (RLKs). Weihua Tang (Plant Gene Expression Center, United States Department of Agriculture/Agricultural Research Service-University of California, Berkeley, Albany, CA) presented characterization of three pollen-specific leucine-rich repeat RLKs (designated PRKs) from tomato. Pollen tube growth is likely to involve diverse communications between the pollen tube and the pistil or between pollen tubes. Pollen and pistil cDNA libraries were screened to find upstream or downstream interacting factors using the extracellular or cytoplasmic domain of the PRKs as bait in the yeast two-hybrid system. Screening with the cytoplasmic domain yielded many unknown proteins, including a potentially highly phosphorylated protein as well as a kinase and a phosphatase as candidates for downstream factors. The bait from the extracellular domain raised leucine-rich repeat proteins, small cysteine-rich proteins, and cell wall–remodeling proteins. The presence of multiple PRKs and multiple putative ligands suggests that there might be timely and spatially combinatorial interactions between the different PRKs and the extracellular ligands from the pollen or the pistil.

Wall-associated kinases (WAKs) are another type of RLK, with epidermal growth factor–like domains in the extracellular regions that are connected covalently to the cell wall pectin (Wagner and Kohorn, 2001). Bruce Kohorn (Bowdoin College, Brunswick, ME) and Zheng Hui He (San Francisco State University, CA) provided an update of their ongoing investigation of WAKs. Five WAK genes have been identified in Arabidopsis, and 25 other WAK-like genes are located as tandem repeats. WAKs are expressed throughout development in a variety of expanding cell types. Antisense experiments using a dexamethasone-inducible promoter showed that WAKs are required for cell expansion (Lally et al., 2001; Wagner and Kohorn, 2001). Inducible antisense inhibition of WAK2 or WAK4 led to a reduction in all WAKs and arrested plant growth at whatever stage of development the steroid inducer was applied. WAKs may function downstream of the growth hormone actions during cell expansion because growth arrest could not be rescued by auxin or GA. Preliminary evidence also suggests that some WAKs bind extracellular glycine-rich proteins.

VASCULAR SYSTEM BIOLOGY

Within the xylem of vascular plants, tracheary elements are responsible for water conduction and are key to the support of the plant body. Tracheary elements also are valuable model cells for investigating the regulation of programmed cell death. Eric Beers (Virginia Polytechnic Institute and State University, Blacksburg) reported on the characterization of a xylem-specific protease, XCP1, that was cloned from an Arabidopsis xylem cDNA library (Zhao et al., 2000). XCP1 appears to be a papain ortholog and is localized specifically in the vacuoles of the differentiating tracheary elements. When expressed ec-

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topically by the 35S promoter of *Cauliflower mosaic virus*, XCP1 caused seedling mortality, stunting, and early senescence of transgenic plants.

Phloem not only conducts the photosynthetic products but also functions as an information superhighway by translocating hormones and macromolecules such as RNAs and proteins. Byung-Chun Yoo (University of California, Davis) reported on phloem-specific calmodulin-like domain protein kinase 1 (CmPCPK1) from *Cucurbita maxima*. CmPCPK1 showed many aspects of calmodulin-like domain protein kinases, namely, Ca^{2+}-dependent phosphorylation activity, cross-reactivity with calmodulin-like domain protein kinase antibody, and an electrophoretic mobility shift in the presence of Ca^{2+}. A phloem single-stranded RNA binding protein (CmPRBP27) was proposed as a putative substrate of CmPCPK1, suggesting that CmPCPK1 may function to alter RNA–protein interactions in the phloem by its Ca^{2+}-dependent phosphorylation activity.

DEVELOPMENT OF PATTERN

Cris Kuhlemeier (University of Berne, Switzerland) described work his group has undertaken to determine how leaf placement, or phyllotaxy, is regulated. Ablation studies using lasers and hand dissection of the shoot apical meristem (SAM) showed that the epidermis is required for leaf initiation, although an intact epidermis is not required. The central zone that has been shown by other groups to be essential for apical-basal pattern formation is entirely dispensable for leaf initiation. Kuhlemeier's group also has investigated the chemical signal required for leaf positioning. Using tomato meristems, they showed that auxin inhibitors prevented leaf initiation, whereas application of lanolin containing auxin to various regions in the meristem elicited leaf primordia development. In collaboration with Göran Sandberg (University of Umea, Sweden), Kuhlemeier's group dissected 1000 tomato meristems and quantitated auxin content—not a trivial task—giving a first indication that an indoleacetic acid gradient occurs throughout sites of leaf initiation. Work with SAMs of auxin mutants further supports the hypothesis that auxin is required for the establishment of leaf position but not for organ identity (Reinhardt et al., 2000).

Kathy Barton (University of Wisconsin) has focused on the establishment of SAMs, and her work with mutants that do not form normal SAMs or that develop ectopic SAMs is beginning to elucidate the mechanisms of SAM regulation. For example, a dominant mutant called phabulosa exhibits adaxial leaf traits around its circumference. This mutant is affected in a homeodomain transcription factor that appears to bind an unidentified sterol- or lipid-signaling component. Binding of the transcription factor to the ligand is thought to affect its own stability, resulting in polarized distribution in the SAM.

Leaf veins are formed in highly regular and reproducible patterns in higher plants. Francine Carland, a member of Timothy Nelson's laboratory (Yale University), described a study seeking to determine the molecular basis for venation in Arabidopsis. Mutants with irregular venation patterns in cotyledons were identified, and one mutant named *cotyledon venation pattern 1* (*cvp1*) was studied in detail (Carland et al., 1999). This mutant has misshapen, misaligned vascular cells, is affected at an early stage of vascular development, and shows pleiotropic elongation and developmental defects at older stages. The gene affected in this mutant was cloned and encodes a sterol methyltransferase. There are three sterol methyltransferases in the Arabidopsis genome. These enzymes synthesize membrane components that contribute to membrane physical properties. Flux through these pathways also affects the pool size of brassinolide. Although the mutant showed increased levels of campesterol, the precursor to BRs, elongation defects still were observed, and these defects were not rescued by the application of exogenous BR. These data suggest that *CVP1* is involved in the production of a sterol signal unrelated to BR or that the effect of the mutation is caused by sterol-related membrane properties.

Phil Benfey (New York University) discussed radial patterning in the Arabidopsis root. Forward genetics previously allowed Benfey's group to identify two Arabidopsis root radial patterning mutants. The *scarecrow* (*scr*) mutant has a single layer of root cells that have characteristics of both endodermis and cortex cells, whereas a similar single layer in the *short root* (*shr*) mutant shows only cortex characteristics. Both genes have been cloned, and both encode members of the GRAS family of transcription factors (Di Laurenzio et al., 1996; Pysh et al., 1999; Helariutta et al., 2000). Localization studies using GFP fused to SHR or SCR have shown, somewhat unexpectedly, that SHR is transferred from cells in the stele to cells in the endodermis. Once in the endodermal cells, SHR is thought to cause the expression of *SCR* and independently to establish endodermal cell fate (Nakajima et al., 2001). These experiments are establishing a model for the genetic control of radial patterning in roots.

MORE USES FOR TRICHOMES

Humans have long made use of plant trichomes, secretion glands on leaf surfaces that may exude secondary metabolites such as flavor and aromatic compounds. George Wagner (University of Kentucky, Lexington) and colleagues are interested in using tobacco trichomes for metabolic engineering and molecular farming. This group has made a subtracted tobacco trichome cDNA

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library and is using it to identify trichome-specific genes. One such gene, which encodes a cytochrome P450 hydroxylase, was manipulated by antisense and sense cosuppression strategies. The transgenic plants showed a decreased level of a major trichome exudate compound, cembratriene-diol, and an increase in the precursor compound cembratriene-ol (Wang et al., 2001a). This precursor is toxic to aphids. In feeding studies, transgenic plants suffered less colonization by aphids than wild-type plants, and exudate from trichomes had higher aphicidal activity. The group is interested in using the promoter from this trichome-specific gene in other metabolic engineering projects to confer altered chemistry.

MINING NUTRIENTS FROM THE SOIL

Leon Kochian (United States Department of Agriculture/Agricultural Research Service and Cornell University) remarked that he has been using any available molecular tools to study how plants mine nutrients from the soil and that he finds it liberating to approach physiological problems "by any means necessary." Kochian's group has identified genes encoding Zn^{2+} transport proteins, Zn^{2+}-responsive transcription factors, and *cis*-acting regulatory elements in the zinc- and cadmium-hyperaccumulating plant *Thlaspi caerulescens*. Several of these genes were identified by complementation of yeast mutants using a *T. caerulescens* cDNA library. In efforts to understand the molecular basis of aluminum tolerance, Kochian's group recently identified an aluminum-activated anion channel in maize root cell protoplasts that is thought to extrude Al^{3+}-chelating organic acids into the soil. They also are attempting to map genes involved in aluminum tolerance in wheat, sorghum, and Arabidopsis.

Nancy A. Eckardt
News and Reviews Editor

Hyung-Taeg Cho
Postdoctoral Research Associate
Department of Biology
Pennsylvania State University
University Park, PA

Robyn M. Perrin
Graduate Research Assistant
Michigan State University–Department of Energy
Plant Research Laboratory
East Lansing, MI

Matthew R. Willmann
Graduate Research Assistant
Department of Genetics
Harvard Medical School
Boston, MA

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