tative transmembrane Pth11 protein echoes many of the signal transduction themes outlined in the contribution from Dixon et al. that describes a novel MAPK. Above all, it becomes apparent that appressorium functionality in *M. grisea* emanates from the complex interplay of signal transduction motifs that are integrated, often by surprising means, into a highly specific whole. One example of this unexpected complexity is offered in the observation that exogenous cAMP, but not diacylglycerol, restores pathogenicity to *pth11* mutants, whereas either metabolite can restore appressorium formation. In addition, the Pth11 protein appears to promote appressorium differentiation not only in response to a surface providing a hydrophobic environment, but also as cued by cutin monomers, the only known alternative surface-inductive cue. Previously, the two cues had been presumed to operate by unrelated pathways.

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A Weed Reaches New Heights Down Under

More than 400 researchers attended the 10th International Conference on Arabidopsis Research, organized this year for the first time in the Asia Pacific region and southern hemisphere, at the University of Melbourne, Australia (July 3–8, 1999). The conference was noteworthy not only for its venue, but also because of the many exciting new data it encompassed. The culmination of molecular analyses into insights that address plant biology at cellular and organismal levels arose as a definite trend. Arabidopsis researchers once again underscored the particular relevance of

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Arabidopsis both to plant biology as well as to the wider scope of biological science. In his keynote address, Elliot Meyerowitz (Caltech, CA) drew on data from various genome projects to compare the molecular machinery of plants and animals as multicellular organisms. Specifically, he observed the extensive molecular similarities between plants and animals in terms of their nuclear transcriptional regulators, and also pointed out the more modest relatedness between the multicellular kingdoms insofar as the proteins used for signaling at the cell membrane are concerned. Completely distinct protein families become apparent, however, upon consideration of the molecules present at the cell surface and extracellular matrix. In this way, the stage was set for the ensuing 12 sessions covering an array of subspecialties within Arabidopsis research. In this Meeting Report we will highlight some of the presentations contained within these 12 sessions. Abstracts of the presentations can be accessed from the Conference homepage (http://arabidopsis. en-bio. com.au/).

STRESS AND SIGNALING: MORE GENES IN THE HIDDEN PATHS

Several newly identified genes important to the signaling pathways under the control of environmental stimuli were discussed. Carol Anderson (Scripps Institute, CA) reported on the REVEILLE (RVE) family of signaling proteins that carry MYB-like DNA binding domains and undergo circadian cycles of expression. Overexpression of CCA1 (for circadian clock-associated protein [Green and Tobin, 1999]), RVE1, or RVE2, moreover, interferes with the rhythmic expression of CAB2 (for chlorophyll a/b binding protein). Genes involved in another light-induced behavior, phototropism, were discussed by Tatsuya Sakai (Kyoto University, Japan). Specifically, a newly identified root/hypocotyl phototropism gene, *RPT2*, functions downstream of *NON-PHOTOTROPIC HYPOCOTYL1* (*NPH1*), the gene that encodes the blue light receptor also known as phototropin (Ahmad, 1999). *RPT2* encodes a protein with putative phosphorylation sites and a nuclear localization signal. Hye Ryun Woo (POSTECH, Korea) presented ongoing studies of *oresara* (*ore*) mutants, characterized by delayed leaf senescence. Some of the *ORE* genes are involved in a common process of senescence that is promoted by a variety of factors (Weaver et al., 1998), including age, dark, abscisic acid (ABA), ethylene, and jasmonic acid (JA). Other *ORE* genes are specific to the age-dependent pathway of senescence alone.

A variety of genes are induced by abiotic stress factors, such as drought, low temperature, and high salinity. Kazuo Shinozaki (RIKEN, Japan) reported on signal transduction mechanisms that are invoked by drought conditions. The C-repeat/dehydration-responsive element (DRE/CRT) is a well-described, major *cis*-acting element involved in ABA-independent responses to drought and cold. One of the DRE/CRT binding proteins, DREB1/CBF1, functions primarily in the cold response, whereas another binding protein, DREB2, is involved in the drought response. Shinozaki also described transgenic plants that overexpress DREB1A so as to manifest enhanced tolerance to drought, salt, and freezing (Kasuga et al., 1999). A hybrid-type histidine kinase, AtHK1, that complements yeast *sln1* mutants was shown to play a role in the osmosensing process. Responsiveness to heavy-metal stress was investigated by Suk-Bong Ha (University of Melbourne, Australia), who analyzed a cadmium-sensitive mutant (*cad1*). Positional cloning showed *CAD1* to encode a phytochelatin synthase (Ha et al., 1999). Phytochelatin is a cysteine-rich heavy-metal binding peptide. Peter Hunt (CSIRO, Australia) reported on genes for two hemoglobins, AHB1 and AHB2 (Trevaskis et al., 1997), that differ from the symbiotic leghemoglobins in that they cannot donate oxygen. The two hemoglobins may have different functions as judged from their biochemical characteristics and expression patterns.

The variety of molecules that participate in phytohormone signal transduction pathways was also illustrated. Joe Ecker (University of Pennsylvania, PA) summarized molecular processes of ethylene signal transduction and introduced two newly isolated genes. *RESPONSIVE TO ANTAGONIST1* (*RAN1*) encodes a protein similar to the copper-transporting P-type ATPases and supports the function of ethylene receptors by delivering the essential copper ion (Hirayama et al., 1999). *ETHYLENE INSENSITIVE2* (*EIN2*) encodes a dimorphic protein with 12 transmembrane regions and a coiled-coil domain. Overexpression of the C-terminal domain is sufficient to evoke constitutive activation of ethylene responsiveness and restores *ein2* mutants with responsiveness to JA and paraquat-induced oxygen radicals (Alanso et al., 1999). EIN2 thus works as a mediator common to the transduction of multiple volatile signals. New players in the gibberellin (GA) signal transduction pathways were revealed by Tai-ping Sun (Duke University, NC). A negative regulator of GA signal transduction, RGA, bears sequence similarity with the products of the *GA INSENSITIVE* (*GAI*) and *SCARECROW* (*SCR*) genes (Pysh et al., 1999). Proteins of the GRAS (for GAI, RGA, and SCR) family have three conserved domains. The DELLA domain is important for the inactivation of RGA and GAI by the GA signal. Another negative regulator of GA signaling, SPY (the product of the *SPINDLY* locus; Robertson et al., 1998), resembles animal O-linked *N*-acetylglucosamine transferases. Based on the phenotypes of SPY overexpression

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lines, Stephen Swain (University of Minnesota, MN) suggested that SPY interacts with RGA and/or GAI and negatively regulates GA signal transduction. There is thus cross talk between different signal transduction pathways. Casper Huijser (University of Utrecht, The Netherlands) reported the involvement of ABA and *ABI* (for *ABA INSENSITIVE*) in sugar sensing. The sugar-sensing mutant *sun6* (Dijkwel et al., 1997) is insensitive to ABA and related to one of the *abi* mutants. Yuji Kamiya (RIKEN, Japan) reported on the phytochrome regulation of gibberellin 3-beta-hydroxylase genes during germination.

Additional molecules involved in signal perception were discussed. June Myoung Kwak (Pohang University of Science & Technology, Korea) reported that a Brassica homolog of the mammalian glutamate receptor, BnGluR1, is required for light- and auxin-induced stomatal opening. Transgenic tobacco plants expressing an antisense gene for BnGluR1 are drought tolerant and have reduced stomatal conductance under well-watered conditions. The role of cuticle in epidermal cell interactions was discussed by Robert Pruitt (Harvard University, MA; Lolle et al., 1997). He isolated a series of organ-fusion mutants, and showed that many genes are involved in cuticle formation and permeability.

METABOLISM, TRANSPORT, AND TRAFFICKING

Proteins destined for plant vacuoles contain sorting signals by which they are excluded from the default secretory pathway that otherwise delivers proteins to the cell surface (Sanderfoot and Raikhel, 1999). Natasha Raikhel and colleagues (Michigan State University, MI) have identified several components of the machinery that direct protein cargo with an N-terminal propeptide (NTPP) signal sequence to the vacuole. In particular, Raikhel et al. have found that AtELP (for Arabidopsis thaliana epidermal growth factor receptor-like protein), a protein that shares many common features with mammalian and yeast transmembrane cargo receptors, is located at the *trans*-Golgi network (TGN), where it interacts with the TGN-specific AP-1 adaptor protein–clathrin complex. Furthermore, consistent with a role in trafficking vacuolar cargo, it was found that AtELP colocalizes with a t-SNARE (AtPEP12p) previously assigned to the prevacuolar compartment. Interestingly, AtVPS45, a peripheral membrane protein homologous to a yeast protein required in vacuolar targeting, cofractionates with AtELP but not with AtPEP12p. Characterization of the connections between these and other components should result in a more detailed understanding of the plant secretory machinery.

Plants are a principal source of iron in most diets, and their global importance in this regard is reflected by the fact that iron deficiency afflicts 2.7 billion people worldwide. Mary-Lou Guerinot (Dartmouth College, NH) reported on the cloning of a root ferric reductase gene, *FRO2*, whose activity is required for the absorption of iron from the soil (Robinson et al., 1999). Guerinot described amino acid substitutions of the putative iron transporter (IRT1) that alter substrate recognition and transport. Such information may allow researchers to construct transgenic plants that can specifically accumulate one cation while excluding others.

Plants have three distinct compartments in which translation takes place (cytosol, mitochondria, and chloroplasts). Aminoacyl-tRNA synthetases (aaRSs) and tRNAs are thus essential components within three biochemical contexts. Peeters and coworkers (INRA, France) have set up a database that describes all known tRNAs and aaRSs from Arabidopsis (www.inra.fr/USER/PRODUCTIONS/BDD/TAARSAT/). Upon studying the distribution of tRNAs and aaRSs among the three distinct compartments, Peeters et al. realized that existing prediction programs, although otherwise useful, are poor at distinguishing mitochondrial and plastid targeting sequences. The investigators have therefore designed a new, neural-network-based program (named "Predator") that they have optimized for the prediction of mitochondrial and plastid targeting sequences. The usefulness and accuracy of Predator have been verified using fusions of putative aaRS targeting sequences to the green fluorescent protein (GFP).

Michael Santos (Virginia Polytechnic, VA) described the expression of anti-chalcone synthase and anti-chalcone isomerase antibodies (Pelletier et al., 1999) in the single-chain format (scFv) from the Nissim phage display library and reported on the use of the antibodies to regulate flavonoid biosynthesis in Arabidopsis. Preliminary results from HPLC profiling of transgenic seedlings showed significant alterations of flavonoid levels, indicating that the phage-derived antibodies can provide a feasible approach for disrupting or redirecting metabolic processes in the plant cytosol.

In Arabidopsis, very-long-chain fatty acids (VLCFAs; $>$C20) are found in seed oils and are the precursors of cuticular waxes. Anthony Millar (University of British Columbia, Canada) reported that the first enzyme of the pathway, an epidermis-specific condensing enzyme, determines both the amounts and chain lengths of fatty acids produced. The gene for a cuticular wax-specific condensing enzyme (*CUT1*) has been isolated (Millar et al., 1999). Cosuppression of *CUT1* results in a waxless phenotype and conditional male sterility. These results highlight the importance of specific condensing enzymes for VLCFA biosynthetic pathways and indicate that they will be key targets for metabolic engineering.

To date, 13 different plasma membrane K^+ transporter genes in Arabi-

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dopsis have been identified. Whitney Robertson (University of Wisconsin, WI) described a PCR-based reverse-genetic screen to detect T-DNA insertions in eight of twelve analyzed K^+ transporter genes. The knockout plants will be analyzed biochemically to assess the relative contribution of each gene to K^+ transport in Arabidopsis.

PLANT PATHOGEN INTERACTIONS: MANY PATHS LEAD TO RESISTANCE

Resistance (R) genes in Arabidopsis encode proteins with a central nucleotide binding site (NBS) and C-terminal leucine-rich repeats (LRRs). Class I (or TIR-NBS-LRR) proteins have N termini that are similar to the cytoplasmic domains of the Drosophila Toll and mammalian interleukin-1 transmembrane receptors, and Class II (or LZ-NBS-LRR) proteins possess putative N-terminal leucine zipper domains. Jane Parker and coworkers (John Innes Centre, UK) reported that resistance conferred by class I genes is dependent on a gene called EDS1 (for enhanced disease susceptibility), whereas that conferred by class II genes depends strongly on a gene called NDR1 (for non-race-specific disease resistance). Thus, distinct resistance pathways operate in Arabidopsis and are likely to be determined by R protein structure rather than by pathogen type. The EDS1 gene was cloned and found to encode a protein with sequence similarities to eukaryotic lipases (Falk et al., 1999). Yeast two-hybrid assays revealed that EDS1 is able to associate with itself, as well as form heterodimers with a second lipase-like protein encoded by the PHYTOALEXIN-DEFICIENT4 (PAD4) gene, originally identified in a different mutational screen for phytoalexin deficiency and cloned previously by Glazebrook et al. (Novartis Agriculture Discovery Institute, CA; see Glazebrook, 1999). The spectrum of resistance affected by the *pad4* and *eds1* mutations is identical, reinforcing the idea that these two lipase-like proteins act in the same disease resistance pathway. Parker hypothesized that their signaling function is achieved through enzymatic hydrolysis of a lipid substrate and that homo- and/or heterodimerization may be crucial for this role.

Systemic acquired resistance (SAR) in plants is transduced by means of salicylic acid (SA). *npr1* mutants (for nonexpresser of pathogenesis-related [*PR*] genes) manifest neither the SA-induced expression of *PR* genes nor acquired resistance to pathogens. To identify additional regulators of SAR, Xinnian Dong and colleagues (Duke University, NC) carried out a genetic screen for suppressors of *npr1-1* so as to recover the recessive *sni1* (suppressor of *npr1-1*, inducible) mutant (Li et al., 1999). The *SNI1* gene product shares moderate sequence homology with the retinoblastoma protein (Rb), a tumor suppressor, and may function as a negative regulator of SAR. In addition, a yeast two-hybrid screen using *NPR1* as bait identified several basic leucine-zipper transcription factors that bind SA response elements of the *PR-1* gene, thus providing a link between *NPR1* and transcriptional regulation.

The systemic movement of plant viruses is an intriguing phenomenon because viruses spread through the infected plant via the symplasmic space, moving from cell to cell through cytoplasmic channels that are normally too small to accommodate their passage (Beachy and Lazarowitz, 1999). Stephen Howell (Boyce Thompson Institute, NY) reported on the identification of Arabidopsis proteins that interact with the movement protein of cauliflower mosaic virus in the yeast two-hybrid system. One of the interacting proteins, called MPI7, is related to an animal protein that interacts with the Rab GTPase and v-SNARE complexes of trafficking vesicles. The MPI7 protein is located in the cytoplasm of uninfected cells and associates with aggregated cytoplasmic structures in cells transfected with the viral movement protein gene.

Uwe Köhler (Cambridge, UK) described an unusual approach for engineering resistance to viruses through the use of *trans*-splicing ribozymes targeted against the viral RNA that encodes the CMV (Cucumber Mosaic Virus) coat protein (Ayre et al., 1999). The strategy aims to construct a functional mRNA for diphtheria toxin specifically within CMV-infected cells. Although the strategy has so far effected specific cell death too late to prevent the spread of CMV, future improvements in the system may overcome this limitation.

EPIGENETICS: THE SILENCE OF THE GENES

The importance of epigenetic mechanisms in the control of many plant processes was illustrated by several speakers. Hervé Vaucheret (INRA, France) demonstrated the mechanistic differences between transcriptional gene silencing (TGS) and post-transcriptional gene silencing (PTGS; Vaucheret et al., 1998). Specifically, *egs* (for enhancer of gene silencing) and *sgs* (for suppressor of gene silencing) mutants affect PTGS, whereas the previously described *ddm* (decrease in DNA methylation), *som* (for somniferous), and *sil* (for altered transgene silencing) mutations affect TGS. At least three *sgs* loci that relieve PTGS were identified. Susceptibility to CMV was enhanced in *sgs* mutants, consistent with the role of gene silencing in plant defense.

PTGS is mediated through RNA degradation. Mark Johnson (Michigan State, MI) used a GUS fusion to a 3′ downstream element (DST) that promotes rapid turnover of those auxin-induced RNAs known as SAURs (for small auxin-up RNAs; Newman et al., 1993), and isolated mutants with high

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GUS expression. Two DST-containing genes that appear to be required for rapid degradation of the SAURs, but not other RNAs, were identified. TGS is likely to be mediated by interactions with chromatin remodeling proteins. Colin MacDougall (Edinburgh, UK) used a yeast two-hybrid screen to show that the polycomb protein CLF (Curly Leaf), a repressor of *AG* (*AGAMOUS*) expression (Kim et al., 1998), interacts with another protein called CLIP (CLF Interacting Protein). Because CLF and CLIP have the same expression pattern, it is likely that they form a complex that limits its expression of *AG* to the last two whorls of the flower.

Perhaps the most surprising findings came from a study on parental gene expression in early embryogenesis. Jean-Philippe Vielle-Calzada (Cold Spring Harbor Lab, NY) reported on collaborative work with Ueli Grossniklaus (FMI, Switzerland) concerning expression of the *MEDEA* gene, which they have previously shown to be required maternally for development of the zygote (Grossniklaus et al., 1998). By a combination of in situ hybridization and reverse transcription-polymerase chain reaction (RT-PCR) they showed that the paternal allele is silenced so that only the maternal allele is expressed in young embryos. This parent-specific silencing (imprinting) is relieved by the *ddm1* mutant, indicating that methylation is involved. Unexpectedly, while attempting to find a positive control for paternal gene expression from among several unrelated genes, they discovered in each case that the paternal allele is suppressed in early embryogenesis. Vielle-Calzada hypothesized that the entire paternal genome might be imprinted, and that paternal gene expression is activated only later in embryogenesis (past the globular stage). For many genes, such late expression may be sufficient to rescue embryos containing a mutant maternal allele. In this way, the effect of imprinting would be manifested only when there is an absolute early requirement for the gene product, as in the case of the *MEDEA* gene.

VEGETATIVE GROWTH: HOW MUCH CELL AUTONOMY?

Genetic regulatory networks that define epidermal cells were discussed by several groups. Martin Hülskamp (Tübingen, Germany) evaluated the role of genes involved in trichome initiation. Whereas the *GL1* (*GLABRA1*) and *TTG* (*TRANSPARENT TESTA GLABRA*) genes appear to encode positive regulators of trichome development, TRY (TRYPTICHON) and CPC (CAPRICE) act as repressors of *GL1* and *TTG* (Schnittger et al., 1999). Highly clustered trichomes were observed in the *try-cpc* double mutant, suggesting that TRY and CPC may work together to inhibit differentiation of neighboring epidermal cells into trichoblasts. Whereas the *TTG* locus had been reported to encode a WD-40 protein by Amanda Walker (Cambridge, UK; Walker et al., 1997), the *ttg* mutation was complemented by the maize R protein, a basic helix-loop-helix protein. The involvement of an R ortholog in trichome initiation in Arabidopsis has long remained an open question. Thomas Payne (University of Texas at Austin, TX) reported that GL3 could be the elusive R ortholog.

Cell–cell signaling also plays an important role in the differentiation of root hair cells. Kiyotaka Okada (Kyoto University, Japan) showed that CPC represses the expression of GL2, a negative regulator of root hair cells (Wada et al., 1997). Because CPC is preferentially transcribed in non-hair cells, Okada proposed a model whereby CPC moves into hair cells from neighboring non-hair cells so as to repress GL2. Hülskamp proposed that TRY is also involved in the intercellular signaling between the root hair cells and their neighbors. Takashi Hashimoto (NAIST, Japan) reported that the *SPIRAL1* gene (*SPR1*) is required for coordinated cell elongation in root and hypocotyl. Expression of the SPR1 protein in the epidermis and cortex is possibly linked to regulation of the cortical cytoskeleton.

Two groups reported a newly discovered gene family that specifies the abaxial cell fate of lateral organs. A flower development gene, *FILAMENTOUS FLOWER* (*FIL*), was cloned in the Okada laboratory and found to encode a protein with a zinc finger and a domain similar to that required for the DNA binding activity of the high-mobility group proteins (Sawa et al., 1999). John Bowman (University of California at Davis, CA) and David Smyth (Monash University, Australia) cloned a gene that promotes pistil development, *CRABS CRAW* (*CRC*; Bowman and Smyth, 1999). Both *FIL* and *CRC*, members of the *YABBY* gene family, are expressed in the abaxial cells of cotyledons, leaves, and floral organs. Shinichiro Sawa (Tokyo Metropolitan University, Japan) observed the partial conversion of adaxial leaf epidermal cells into an abaxial route of development in transgenic plants carrying the *FIL* gene under control of a 35S promoter. Bowman observed the pronounced abaxialization of leaves in a *fil-yabby3* double knockout mutant, suggesting that *FIL* and *YABBY3* share redundant roles in determining abaxial fates.

A number of presentations focused upon mechanisms of meristematic organization. Thomas Laux (Tübingen University, Germany) reported that *WUSCHEL* (*WUS*) and *ZWILLE* (*ZLL*) are expressed from early embryonic through adult developmental stages in cells that underlie the meristematic stem cells (Mayer et al., 1998; Moussian et al., 1998). This finding indicates that cell fate within the shoot apical meristem (SAM) is informed by signals from the underlying cells. Rudiger Simon

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(University of Cologne, Germany) reported that *CLAVATA3* (*CLV3*) encodes a small protein that may be a ligand for the heterodimeric membrane-bound receptor kinase that is encoded by *CLV1* and possibly by *CLV2* (Fletcher et al., 1999). Specifically, a 35S promoter-driven *CLV3* construct reduced the size of the SAM in a wild-type background, but failed to limit the size of the SAM when expressed in a *clv1* mutant background, thereby indicating CLV3 to be a ligand for the CLV1 protein. Philip Benfey (New York University, NY) showed that root radial development is dependent on differentiation cues provided by *SCARECROW* (*SCR*) and *SHORT ROOT* (*SHR*) in the cortex/endodermis (Benfey, 1999). *SCR* is expressed in the endodermis and cortex/endodermis initials, whereas *SHR* expression occurs in the central cylinder, which suggests that *SHR* does not act in a cell autonomous manner. Dimitris Beis (Utrecht University, The Netherlands) reported that ablation of cells from the quiescent center (QC) of the root meristem and addition of auxin polar transport inhibitors ectopically induced QC and columella identity markers, indicating that pattern and polarity in roots is maintained by auxin distribution.

FLORAL INDUCTION AND DEVELOPMENT: BEFORE ABC AND AFTER

Floral induction was one of the hottest topics of the meeting. Cloning and molecular characterization of genes representative of various functional groups confirmed many aspects of previous genetic models of floral induction. Wim Soppe (Wageningen University, The Netherlands) discussed the late-flowering gene, *FWA*, of the photoperiodic promotion pathway encoding a homeodomain protein (Levy and Dean, 1998). Interestingly, the *FWA* gene is not expressed in wild type, but is expressed in the *fwa* mutant, possibly as a result of reduced methylation of the promoter. Such a conclusion is supported by the isolation of an allelic mutation from progeny of the low-methylation *ddm1* mutant. Recent progress on the genetics of the vernalization response was summarized by Tony Gendall (John Innes Center, UK). Late-flowering phenotypes resulting from the dominant allele of *FRIGIDA* (*FRI*) can be suppressed by vernalization. The dominant *FRI* allele was found in those ecotypes growing in cold, northern Europe, but not those from warm, southern areas. *FRI* encodes a protein with coiled-coil domains. *VERNALIZATION2* (*VRN2*), modulating the sensitivity of the vernalization response, was shown to encode a putative transcription factor. Another repressor of flowering, *FLF/FLC*, was cloned recently (Michaels and Amasino, 1999; Sheldon et al., 1999). Candice Sheldon (CSIRO, Australia) explained that elevated levels of *FLF/FLC* expression correlate with later flowering time. Sarah Fowler (University of Auckland, New Zealand) discussed the cloning and characterization of the photoperiod-dependent flowering gene, *GIGANTEA* (*GI*). The gene encodes a large protein with putative transmembrane domains, and its expression follows the circadian regulation regimented in part by *LATE ELONGATED HYPOCOTYL* (*LHY*) and *CCA1* (Fowler et al., 1999). The cloning of a flowering-time gene (*FT*), also belonging to the photoperiodic promotion pathway, was reported by Yasushi Kobayashi (Kyoto University, Japan). Longevity of flowering is dependent on gene dosage of *FT*, the product of which is similar to TFL1 (for terminal flowering protein 1). *FT* functions downstream of *CONSTANS* (*CO*) and its expression is upregulated by *CO.* Doris Wagner (Caltech, CA) used a steroid-inducible activation system to demonstrate that the LEAFY protein directly activates transcription of *APETELA1* (*AP1*). Koji Goto (Kyoto University, Japan) examined the cell–cell nonautonomous action of some floral homeotic genes. A transgenic approach, in which an L1-specific (outermost layer) promoter was used to drive the *PISTILLATA* (*PI*) and *AP3* genes, allowed for the partial rescue of *pi* and *ap3* mutations in all three cell layers. When the gene encoding GFP was fused to *PI* or *AP3* under control of the same promoter, however, GFP fluorescence was observed only in the L1 layer. These results suggest that organ identity signals are transmitted from L1 layer to L2 and L3 layers by downstream molecules rather than directly by the activities of PI or AP3.

FRUIT AND SEED DEVELOPMENT: THE RULES OF MULTIPLICATION

Fruit dehiscence and shattering is an important agronomic problem in crops such as canola. Martin Yanofsky (UC San Diego, CA) described the roles of two MADS box genes, *SHATTERPROOF1* (*SHP1*) and *SHATTERPROOF2* (*SHP2*) (formerly called *AGL1* and *AGL5*), in fruit shattering. Whereas the single mutants manifest no phenotype, Yanofsky showed that the *shp1-shp2* double mutants are indehiscent, thereby indicating that *SHP1*/*SHP2* functions are necessary for dehiscence zone formation.

Genetic analyses have led to the identification of most genes that define the ovule development pathway. Charles Gasser (UC Davis, CA) described studies on *INNER NO OUTER* (*INO*), a gene required for the development of the outer integument (Baker et al., 1997). The sequence of *INO* revealed a relationship to the *YABBY* gene family, which includes *CRC* and *FIL*. The expression pattern of *INO* indicates that it may be required for specification of abaxial identity. The question of abax-

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ial-adaxial identity was further elaborated by Yuval Eshed (UC Davis, CA), who described a screen for second-site enhancers of the *crc* mutation. Mutations at three loci, designated *GYMNOS*, *AKETHI* and *KANADI*, lead to the ectopic formation of adaxial tissues on the carpels. The *GYMNOS* gene has been cloned and found to encode a homolog of the yeast SNF1 chromatin remodeling protein.

Mutations in three genes, *FERTILIZATION-INDEPENDENT SEED1*/*MEDEA* (*FIS1*/*MEDEA*), *FIS2*, and *FIS3/FERTILIZATION-INDEPENDENT ENDOSPERM* (*FIS3*/*FIE*) have been found to result in seed development in the absence of fertilization (see Preuss, 1999). Robert Fischer (UC Berkeley, CA) reported that the *FIE* gene encodes a WD-40 protein, homologous to products of the *MES6* gene of *C. elegans* and the *Extra Sex Combs* (*esc*) gene of Drosophila. The FIE product interacts with the MEDEA/FIS1 product in a yeast two-hybrid assay. He also provided data indicating that paternally derived *MEDEA*/*FIS1* is active in later embryos but is inactive in the endosperm (see also section on Epigenetics, above). Abed Chaudhury (CSIRO, Australia) reported that *FIS2* encodes a putative zinc-finger transcription factor. Using a promoter fusion to GUS, FIS2 expression is first detected in the embryo sac in the two central cell nuclei prior to their fusion. Following pollination, FIS2 expression persists through the free nuclear endosperm stage and is mostly abolished in the endosperm at cellularization. *FIS3* was also cloned and shown to be allelic to *FIE*. Chaudhury also reported that FIS3 interacts with FIS1/MEDEA in the yeast two-hybrid assay, and partially defined the interacting domains of FIS1.

Genes that act very early during embryogenesis would provide clues to the initial events of embryo development. Sacco de Vries (Wageningen Agricultural University, The Netherlands) reported that expression of the *AtSERK* gene, which encodes an LRR-type transmembrane somatic embryogenesis receptor-like kinase, is first detected in the developing ovule primordia after meiosis (Schmidt et al., 1997). AtSERK is present in the female gametophyte and persists after fertilization in all cells of the embryo, including the suspensor, up to the eight-cell stage. Overexpression of *AtSERK* with the 35S promoter results in an increased competence to somatic embryogenesis, suggesting that *AtSERK* may play a role in both zygotic and somatic embyrogenesis.

GENOME STRUCTURE AND FUNCTION: ON THE EVE OF THE COMPLETE SEQUENCE

Arabidopsis will be the first plant to have its entire genome sequenced, with completion of the project now certain to occur in the year 2000. The rapid progress of the Arabidopsis genome sequencing project was the subject of presentations by Satoshi Tabata (Kazusa Institute, Japan), Robin Buell (TIGR, USA) and W. Richard McCombie (Cold Spring Harbor Lab, NY). Tabata stated that 16 Mbp of chromosome 5 and 5 Mbp of chromosome 3 had been sequenced. Analysis of 11 Mbp on chromosome 5 revealed 2700 genes, with a density of 1 gene/5 kb. A new database, called Arabidopsis Genome Displayer (AGD), provides annotated sequences for the whole genome (www.kazusa.or.jp/arabi/displayer/). The 160-kbp chloroplast genome has also been sequenced, and found to encode 113 proteins. Buell reported that the sequence of chromosome 2 was almost completed with remaining gaps in the centromere and telomere regions. An Arabidopsis Genome Annotation Database (AGAD; www.tigr.org/tdb/at/at.html) has been developed at TIGR and allows searches of all the available Arabidopsis sequence data for any type of protein or gene sequence. The database includes predicted genes and homology search results in the annotation, and importantly, it will be updated regularly. McCombie reported that the Cold Spring Harbor consortium and the European ESSA project have almost completed the sequence of chromosome 4. Within the 17-Mbp of chromosome 4 so far sequenced, 3400 genes are predicted.

Whereas the public sequencing projects have selected the Columbia ecotype, Rounsley (Cereon Genomics, MA) described a parallel effort to sequence the genome of the Landsberg *erecta* ecotype, with the object of identifying polymorphisms to facilitate mapping and map-based cloning. Rounsley reported the shot-gun sequencing of nearly 70% of the genome and identification of nearly 7000 SNPs (Single Nucleotide Polymorphisms) and 4500 Indels (Insertions and Deletions) between the Col and L*er* genomes. The combined density of SNPs and Indels is 1 per 6kbp, indicating that it should be possible to find such markers for most genes in Arabidopsis.

The identification of all the estimated 25,000 genes in Arabidopsis from sequencing projects will be followed by intense efforts to deduce their biological functions. Insertional mutagenesis to generate gene knockouts is one approach to this task, illustrated by talks from David Bouchet (INRA, France) and Serguei Parinov (IMA, Singapore). Bouchet described a successful PCR-based strategy using pools of DNA from a collection of 55,000 T-DNA insertions generated at INRA. From this population of insertions, only 3% resulted in obvious visible phenotypes, highlighting the importance of designing screens for subtle or conditional phenotypes. Parinov described a random sequencing strategy used to generate a flanking-sequence database from a collection of *Ds* insertions generated at IMA. This information can be used

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to rapidly identify a knockout in a given gene by a computer search. Nearly half of 1000 insertions analyzed fell within sequenced regions of the genome, and of these, 70% occurred in known or predicted genes. An unexpected finding was that *Ds* insertions preferentially occur at chromosomal regions flanking the nucleolar organizing regions but avoid rDNA, probably reflecting differences in chromatin configurations in these regions.

A different approach to deducing function is to determine under what conditions a gene will be expressed. Microarrays are powerful tools for this purpose, as illustrated by talks by Iain Wilson (Carnegie Institution, MI) and Feng Zheng (Novartis, USA). The Carnegie group used microarrays consisting of 2200 cDNAs, and cluster analysis, a method first used in yeast, to determine sets of genes that are induced or suppressed during infection by powdery mildew. A comparison of resistant and susceptible plants was performed using RNA isolated subsequent to powdery mildew infection from near-isogenic lines for the *RPW10* resistance locus. Using a twofold threshold, 45 genes were found to be induced specifically in resistant plants, whereas 90 genes were induced in susceptible plants alone. Similarly, Zheng described a microarray of 10,000 Arabidopsis expressed sequence tags which was used to identify genes that are induced or suppressed following treatment with the herbicide hydantocidin.

Although microarrays will be the system of choice in the future, they are expensive and currently beyond the reach of most laboratories. Nina Fedoroff (Penn State, PA) described an alternative approach by performing global expression screens based on the hybridization of subtracted libraries to subtracted and total cDNA. cDNA clones for hundreds of genes induced by ozone or salicylate or during embryogenesis were identified by this approach.

FUTURE PERSPECTIVES

The consensus reached at the Arabidopsis Genome Initiative meeting held during the Conference was that sequencing will be completed in July 2000, with the exception of a few regions including centromere and rDNA repeats. Free access to the sequence data will change the methodology of Arabidopsis research. As discussed in the genomics sessions, new databases and techniques will allow investigators to find new genes and functions rapidly. The extensive understanding of one plant species, *Arabidopsis thaliana*, will undoubtedly stimulate studies in related fields of biology.

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Revisiting and Revising the Self-Incompatibility Genetics of *Phalaris coerulescens*

In the grasses, self-incompatibility is controlled by two unlinked loci, *S* and *Z.* Previously, Li et al. (1994) presented a differential screening technique to identify a putative *S* gene clone from *Phalaris coerulescens.* In this approach, a cDNA library made from mature pollen of *P. coerulescens* was screened to identify sequences showing pollen-specific expression and differences in hybridization intensity when probed with cDNA made from pollen RNA isolated from plants with different *S* or *Z* genotypes. The putative *S* gene clone was named *Bm2.* No *Z* candidate was identified (Li et al., 1994). Several lines of evidence seemed to support *Bm2* as representing *S*: (1) cosegregation of a restriction fragment length polymorphism detected with *Bm2* correlated with the *S* genotype in over 120 plants; (2) gene expression predominantly in mature pollen; and (3) identification of a highly conserved C-terminal catalytic domain and a variable, potentially allelic, N-terminal domain in the deduced protein sequence.

A series of recent experiments, however, has shown that *Bm2* in fact does not represent *S.* The bases for this revised conclusion are: (1) recombination between *Bm2* and *S.* It now appears that *Bm2* may be as much as 2 centimorgans away from *S.* Several recombinant plants have been identified that demonstrate linkage between the *Bm2* and *S* loci; (2) sequencing of *S* homologs from other grasses, including members of the *Phalaris* genus, along with additional alleles of *Phalaris coerulescens*. Most *Bm2*-like sequences encode only a thioredoxin protein and no allelic domain. All mRNAs, moreover, show several in-frame stop codons and an ATG start directly before the thioredoxin domain. Thus, *Bm2* sequences encode a novel thioredoxin, but do not contain a region that could determine allele specificity, as was previously suggested (Li et al., 1994).

In summary, it is now clear that *Bm2* cannot represent *S*. *Bm2* is a gene closely linked to *S* that may be involved in some aspect of the self-incompatibility reaction but is unlikely to be involved in the recognition process per se.

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