

## Meeting report

# A window on the world of plants

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A report on the seventh meeting of the International Society for Plant Molecular Biology (ISPMB), Barcelona, Spain, 23-28 June 2003.

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It was the turn of Barcelona to host the seventh meeting of the International Society for Plant Molecular Biology this year. Traditionally this is the largest 'sciencefest' in the plant biologist's calendar, catering for all fields of plant molecular biology, from the fundamentals of developmental genetics to the applied aspects of crop improvement for developing countries.

The President of ISPMB, David Baulcombe (Sainsbury Laboratory, Norwich, UK), opened the meeting by suggesting that the main difference between plant science now and at the first ISPMB meeting in 1985 is that we can now link mutant phenotypes to gene action. Certainly, the importance of molecular genetics to all areas of plant biology was emphasized throughout the subsequent talks and sessions. New platform technologies, such as those described below, are having a great impact on the pace of scientific discovery in plant molecular biology, a message that was made clear throughout the meeting.

### High-throughput studies of plant biology

Joe Ecker (Salk Institute, San Diego, USA) began the talks on platform technologies with his presentation on whole-genome functional analysis in *Arabidopsis*, including the progress made in gene identification and characterization since the publication of the *Arabidopsis* genome sequence in December 2000. The major part of his presentation described the use of tiling arrays for the *Arabidopsis* genome, which have been developed in association with Affymetrix. The entire *Arabidopsis* genome has now been covered by 25-nucleotide oligonucleotides arrayed on 12

chips, each chip covering 20 Mb of DNA. The oligonucleotides are arrayed in the order in which they occur in the genome, so that hybridization of RNA probes allows domains of gene expression to be visualized across chromosomes. Expression frequency, which depends on the density of the genes, dips markedly in regions close to the centromeres, although there are localized zones with high expression in these regions. From experiments using these chips, it was possible to estimate that a large proportion of the genome expresses antisense transcripts (some 7,500 antisense transcripts were recognized), but it is currently difficult to identify microRNAs (miRNAs) because of problems with labeling very short stretches of RNA. Tiling arrays provide a very powerful tool for extended genome analysis, but given their high costs, they will probably remain the province of a few well-resourced pioneers.

Metabolic profiling is another platform technology poised to increase our abilities to study plants and also, hopefully, our understanding of plant biology. Lothar Willmitzer (Max Planck Institute for Molecular Plant Physiology, Golm, Germany) presented the state of the art in plant metabolomics. He described the establishment of databases for metabolite identification for tomato, potato, tobacco, *Arabidopsis*, yeast and other species. With gas-chromatography time-of-flight mass spectrometry (GC-TOF), more than 1,000 metabolites can now be separated and quantified, and more than 200 individual metabolites are known. For *Arabidopsis* leaves, more than 400 metabolites can be identified by liquid chromatography plus mass spectrometry (LC-MS), but quantification by this technique is less accurate. With principal component analysis of the data, ecotypes of *Arabidopsis* can now be separated on the basis of their metabolites. The technology can be taken further by linking differences in metabolite profiles to differences in transcript profiles. Such associations will allow identification not only of expected links but also of novel correlations; for example, Willmitzer has found links between changes in succinyl-CoA

synthase expression and  $\alpha$ -tocopherol levels. Additionally, it is now possible to model non-linear changes in metabolite levels in response to changes in gene expression. Plant physiologists will have an enormous amount to do in the near future, trying to map out the metabolic routes that link causes to their effects. Hopefully, it may also be possible to use this technology in a predictive way to model how to engineer desirable metabolic traits in crop plants.

Detlef Weigel (Max Planck Institute for Developmental Biology, Tübingen, Germany) described the effect on leaf development of misexpression of a miRNA (*jawi*) and gave several examples of genes that are probably targets for silencing by this miRNA. Members of a subfamily of the *TCP* family of genes, encoding transcription factors, have sequences homologous to the miRNA, and the transcript of at least one member appears to be modified by ectopic expression of the miRNA. Weigel claimed to provide the first demonstration of miRNA regulation in plants, but this claim may have to be tempered as a presentation on control of flowering by miRNA was given by Milo Aukerman (Dupont, Crop Genetics, Delaware, USA) a few days earlier at the *Arabidopsis* Meeting in Madison. Weigel can take comfort, however, from the fact that his data are strengthened by independent reports of the importance of miRNAs in plants.

Norio Murata (National Institute for Basic Biology, Okazaki, Japan) described a systematic approach to understanding environmental sensing in cyanobacteria. The ease and simplicity of systematic gene knockouts and their analysis in bacteria contrasts greatly with the more complex approaches and analysis needed for higher plants. The cyanobacterium *Synechocystis* responds to stresses - osmotic stress, cold, heat, visible light, ultraviolet light, salt, oxidative stress, metal ions, anions, and extremes of pH - through simple pathways, in each case involving a sensor, signal transduction, transcription factors that regulate gene expression, and changing levels of mRNA and, consequently, of response proteins. Each response pathway has been analyzed using the 'cyanochip' of *Synechocystis* genes. Under salt stress, for example, 200 genes are upregulated and 200 genes are downregulated; of these, about 50% encode proteins of known function. Under osmotic stress, some of the genes that are induced or repressed are the same genes that respond to salt stress, whereas others are different. The outcome of this systematic approach is that each histidine-kinase signal transducer can be shown to sense one or more specific environmental conditions and the sensors, in turn, signal to induce specific responses. This provides a powerful conceptual framework to use for the analysis of the responses of higher plants to abiotic stresses.

### The genetic analysis of signaling in plants

Joanne Chory (Salk Institute, San Diego, USA) focused on the processes of perception and signaling in response to

brassinosteroid hormones. All the steps in this signal transduction pathway have been identified by genetic analysis, showing the power of this approach to the field of plant hormone signaling, a field that was barely recognized ten years ago. The plant hormone brassinolide is detected by a leucine-rich-repeat-containing receptor-like kinase (LRR RLK) BRI-1. Three other similar LRR RLKs are encoded by the *Arabidopsis* genome: BRL-1, BRL-2 and BRL-3. BRL-1 and BRL-3 bind brassinolide with high affinity, and mutation of BRL-1 affects development of vascular tissue, increasing the proportion of phloem and decreasing the proportion of xylem. BRI-1 is thought to interact with another LRR RLK protein, BAK1, and perception of brassinolide results in the phosphorylation of both proteins, which in turn inhibits the activity of BIN1 (encoding glycogen kinase I). This prevents phosphorylation of two related proteins, BZR1 and BES1, an inhibition that is augmented by the activity of a phosphatase (BSU1). When BZR1 and BES1 are not phosphorylated they accumulate in the nucleus, where they activate transcription factors such as BIM1 to invoke the transcriptional response to brassinosteroid. Another gene, BAF1, inhibits the activity of BES1 by targeting it to the proteasome for degradation. This talk illustrated well how much understanding can be gained from focused genetic analysis.

The theme of signaling was neatly developed by a talk by Jeff Dangl (University of North Carolina, Chapel Hill, USA) on signaling in plant disease and disease resistance. The gene-for-gene system of resistance to biotic stress involves a large number of *R* genes, which encode proteins that have LRR regions and are involved in the recognition of effector molecules (ligands) secreted by pathogens. *Pseudomonas syringae* var tomato, an effective pathogen of *Arabidopsis*, produces only about 30 types of effector; the 'ligand universe' is thus small relative to the number of potential receptors. There is only about 50% overlap between effector types from different pathogenic *Pseudomonas* species, however; this may explain, in part, the large pool of receptors available. Dangl and Jonathan Jones (Sainsbury Laboratory, Norwich, UK) have suggested that the normal function of *R* genes is to guard vital cellular machinery in the plant. This model predicts that several effectors may be recognized by the same *R* protein, and multiple *R* proteins may associate with a single effector to limit its destructive capabilities. Dangl described some of the molecular constituents of this defensive signaling complex in *Arabidopsis*, including the central role of the RIN4 protein and the action of two LRR proteins operating in series around RIN4 to elicit defense responses in response to effector secretion.

The EMBO Lecture, given by Chris Lamb (John Innes Centre, Norwich, UK), continued the theme of signaling in response to biotic challenge. Systemic acquired resistance (SAR) arises when infection of one area of plant tissue invokes a systemic response in other parts of the plant that provides resistance to subsequent re-infection. SAR involves

both an inducing signal and a systemic signal. An *Arabidopsis* mutant defective in induced resistance to fungal elicitors, *dir1*, affects a gene encoding a lipid-transfer protein. DIR1 provides a lipid signal in response to infection; this signal moves out of the leaf through the phloem to induce resistance responses elsewhere. Generation of the defense response in *Arabidopsis* requires another gene, *CDR1*, which encodes an aspartate protease probably involved in producing a small, mobile peptide signal that induces resistance.

Paul Schulze-Lefert (Max Planck Institute for Plant Breeding Research, Köln, Germany) continued the discussion of resistance by emphasising the importance of non-host resistance (the mechanisms by which most plants are resistant to most pathogens). When barley mildew grows on *Arabidopsis* leaves, it does not infect them and the cells attacked remain alive, although the fungus initiates penetration and promotes localized cell-wall remodeling. Genes required for resistance to penetration include *PEN1*, encoding a syntaxin, and *PEN2*, encoding a polysaccharide hydrolase, both of which are involved in cell-wall synthesis. In non-host resistance, extra secretion of cell-wall material around abortive penetration sites is observed, and the fusion of vesicles carrying wall material to the plasma membrane is positively correlated with resistance to penetration. The plant genes involved in resistance are also involved in vesicle tethering and vesicle-membrane fusion to produce this additional wall material.

### The genetic analysis of plant cell walls

Chris Somerville (Carnegie Institute of Washington, Stanford, USA) reviewed the way in which molecular genetics has opened up the understanding of cellulose synthesis over the past five years. The mutational approach is providing insight into less well-understood cell-wall constituents such as cell-wall proteins. Glycosylphosphatidyl-inositol (GPI) anchors facilitate the localization of cell-wall proteins, and the *peanut* mutants of *Arabidopsis* are defective in the attachment of GPI-anchored proteins to membranes. These mutants are embryonic lethals, showing the importance of the wall proteins to wall assembly and plant development in its entirety. In the various *peanut* mutants, arabinogalacturanan proteins (AGPs) are not exported to the wall because of the failure of their GPI anchors. In the *peanut1* mutant, cellulose synthesis is also disrupted. Another cell-wall protein with a GPI anchor is pectate lyase, which hydrolyzes pectin in the primary cell walls. Surprisingly, a mutation in the gene encoding this protein (*pmr6*) confers resistance to powdery mildew in *Arabidopsis*. Somerville suggested that N-linked glycans are normally synthesized and exported to the wall, then partially hydrolyzed by pectate lyase in the wall, and subsequently reused as part of the normal process of wall synthesis. During the turnover of the glycans, oligosaccharides are released, and these are probably used as signals for pathogenic growth of the mildew fungus. If pectate lyase is non-functional, such

signals may not be produced or may be modified, resulting in resistance to infection. If shown to be correct, this model would revolutionize thinking on the assembly and modification of plant cell walls.

The specificity and control exercised over cell-wall glycoprotein synthesis were highlighted by Georg Siefert (John Innes Centre, Norwich, UK). The *Arabidopsis* root hair defective 1 gene (*RHD1*) encodes a UDP glucose-4-epimerase (UGE) required for the synthesis of the UDP galactose that is used for the synthesis of hemicelluloses and AGPs. In *rhd1* mutants, root hairs do not form, and the synthesis of an AGP called LM2 is affected specifically. The *rhd1* phenotype suggests that there is a specific association between the epimerase encoded by *rhd1* (UGE4) and the glycosyl transferases leading to AGP synthesis. If *rhd1* roots are treated with ethylene, the root-hair-defective phenotype is lost. Following ethylene treatment, galactose is synthesized via UGE1 and UGE2, bypassing the block in UGE4.

### Control of plant gene expression by chromatin modification and remodeling

Another theme of the meeting was the importance of chromatin modification and remodeling, particularly in controlling growth and development in response to environmental factors. Jose Martinez-Zapater (Centro Nacional de Biotecnología, Madrid, Spain) described the *Arabidopsis* flowering time gene *FVE*. Mutations in *FVE* delay plant development and cause late flowering; this phenotype can be corrected by exposure to low temperatures (vernalization). The *FVE* protein has homology to mammalian retinoblastoma-associated proteins, which are involved in chromatin assembly, modification and remodeling. The pathways to flowering involving vernalization act through the floral repressor *FLC*, and *FVE* is proposed to function by transcriptional repression of *FLC* through histone deacetylase complexes.

Moving on to translational and post-translational regulation of gene expression, Mike Thomashow (Michigan State University, East Lansing, USA) described how cold-responsive *COR* genes are regulated by genes involved in chromatin remodeling in *Arabidopsis*. On exposure to cold, the CRT/DRE binding factor (CBF) transcription factors bind to an element ('CRT/DRE') in the promoters of *COR* genes and induce freezing tolerance. CBF1 has an activator region that recruits complexes containing histone acetyltransferases (HATs) to the promoters of target genes. Transcriptional activation by CBF1 may work in a similar way to that seen in yeast, through *A. thaliana* homologs of components of the yeast SAGA and ADA HAT complexes. *CBF* genes are themselves induced by the transcription factor inducer of CBF expression (ICE). Thomashow suggested that there are additional pathways for the cold response, because expression of other transcription factors is observed in response to cold in microarray experiments.

## The genetic analysis of plant development

Philip Benfey (Duke University, North Carolina, USA) started with a conceptually straightforward approach to the topic of axis formation and cellular specification in the developing root. He concentrated on the action of two genes in determining cell division patterns and cell identity: *SHORTROOT* (*SHR*) and *SCARECROW* (*SCR*), which encode transcription factors of the GRAS family. *SHR* directs the asymmetric cell division that gives rise to the parenchyma tissue of the root. It also specifies the endodermis, but *SHR* is not expressed in the cells that become the endodermis. Instead, *SHR* protein moves from the stele at the centre of the root into the cells one cell-layer outwards, to specify the endodermis. In these cells, *SHR* activates *SCR* expression. Protein turnover is important in limiting the effects of *SHR* to the cells that will become the endodermis; *SHR* protein that is not localized in the nucleus is rapidly degraded. Extending from this genetic analysis of root patterning and cellular specification, Benfey described a method for transcript analysis of specific cell types in roots. Using green fluorescent protein (GFP) to mark specific cell types by expression from tissue-specific promoters, root cells were separated by making protoplasts and then sorted into specific tissue types by a cell sorter measuring fluorescence. In this way, five populations of cells were defined radially and three longitudinally along the root. Of the 10,000 genes expressed in *Arabidopsis* roots, 5,700 are expressed in a tissue-specific manner of which only 400 are induced by the protoplasting itself. Using these tissue-specific transcript profiles, transcription networks involved in root morphogenesis and root-cell specification are being defined, linking back to the activities of master genes such as *SHR* and *SCR*.

Throughout the meeting, the newly recognized importance of directed protein turnover in controlling signaling and responses was made clear. The role of protein turnover in three signaling pathways was also the focal point of the keynote address presented by Nam-Hai Chua (Rockefeller University, New York, USA). Chua described the role of ubiquitin-targeted degradation pathways in signaling triggered by auxin, phytochrome A and abscisic acid (ABA). In auxin signaling, protein turnover has a significant regulatory role in the removal of the negatively auxin responsive transcription factors (AUX/IAA proteins), following the auxin signal. Removal of these proteins results in the activation of previously repressed transcription factors that can then invoke transcriptional responses. Ubiquitination also negatively regulates the transcription factor NAC1, a positive regulator of auxin responses. In the absence of the phytochrome A signal, the COP1 protein moves to the nucleus in the dark and targets a Myb family transcription factor, LAF1, for degradation. In ABA signaling a protein, AFP, provides a molecular escort for the transcription factor ABI5, targeting it for degradation by ubiquitination. ABI5 operates at an environmental check point in the growth of plants. If the plant is stressed, ABA is induced, and this

signals to induce ABI5 activity and so to invoke transcriptional responses. AFP negatively regulates ABI5 and so modulates the ABA signaling pathway.

## Plant evolution and biodiversity

Alain Charcosset (Institut National de la Recherche Agronomique, Gif sur Yvette, France) presented a fascinating talk on the origins of the molecular diversity of European maize. A large number of European and American maize populations were examined using simple sequence repeat markers. A correlation was observed between specific European lines and lines originating from particular areas in the Americas; maize grown in southern Spain comes mostly from the Caribbean, whereas northern European maize originates in the northeastern USA (Northern Flint). It might have been expected that most European maize would be similar to Spanish maize, having been brought back by Columbus and subsequently spread, but maize with Northern Flint characteristics was commonly observed in Germany in the 1540s, suggesting that maize was independently introduced into northern and southern Europe.

Olivier Panaud (University of Paris-sud, Orsay, France) described the origins of domesticated rice (*Oryza sativa*). From a comparison of sequence data from *japonica* and *indica* rice varieties, the history of retrotransposon transpositions has been used to date when the cultivars diverged. These data showed that the *japonica* and *indica* cultivars diverged 1-2 million years ago, much earlier than domestication, which occurred about 10,000 years ago. These domesticated varieties are, therefore, polyphyletic in origin, having diverged long before either was domesticated or, presumably, before they were bred for their desirable traits.

The ISPMB meetings provide a magnificent forum for demonstrating the breadth of research approaches available in plant sciences. The new platform technologies presented and the data they produce are inevitably going to become ever bigger components of all plant research. For younger scientists the meetings offer a truly impressive shop window for all of the research options available within plant molecular biology. Twenty years after its inception, the future for ISPMB looks bright, and we are looking forward to the eighth meeting in Adelaide, Australia, in 2006.