

## P8—GENERAL PLANT GENE STRUCTURE AND FUNCTION

Organised by M. Holdsworth and K. Lindsey for the Plant Gene Structure and Function Group

### P8.1—Laser-capture microdissection to analyse gene transcription in *Arabidopsis* embryos

S.A. Casson (University of Durham); M.W. Spencer; K. Lindsey

The basic body plan of the *Arabidopsis* seedling is established during embryogenesis. Although the histology of this process has been well characterised, as yet we have only a limited understanding of the genes and transcriptional changes required for the establishment of embryo pattern, morphogenesis and cell differentiation. Microarray analysis has greatly facilitated our ability to examine changes in transcriptomes, but the small size and poor accessibility of *Arabidopsis* embryos has made such analysis difficult. Furthermore, due to these technical difficulties, analysis of specific zones or cell types of young embryos has been almost impossible. Laser Capture Microdissection (LCM) has simplified the problem of separating specific cells from complex tissues. In essence, LCM involves cutting histological sections of the tissue of interest and then targeting the cells of interest with a laser, allowing for their removal from the surrounding tissue. RNA can then be purified from these cells and subjected to analysis. It is our aim to use LCM to examine transcriptome changes between different zones of the embryo.

### P8.2—Use of the GAL4 GFP enhancer trap system to identify genes involved in guard cell function

A.J. Baker, S.W. Hou, Department of Plant Sciences, University of Cambridge, UK; R.S. Poethig, University of Pennsylvania, Department of Biology, Plant Science Institute, Philadelphia, Pennsylvania, USA; J. Haseloff and A.A.R. Webb, Department of Plant Sciences, University of Cambridge, UK

Stomatal guard cells are a specialised cell type that control the aperture of the stomatal pore, hence regulating gas exchange between a plant and the atmosphere. Little is known about the genes involved in the specialised function and development of stomata. We are using the GAL4-GFP enhancer trap system to identify genes involved in guard cell function and/or development. Genes flanking T-DNA inserts driving guard cell GFP expression are predicted to be involved in the specialised function and/or development of the guard cell. 22 lines with GFP expression in the guard cell were identified from the GAL4-GFP electronic databases. Southern

blots identified single insert lines in the guard cell enhancer trap population and further members of this library were reduced to single T-DNA inserts by segregation following back-crossing to the wild-type background. Thermal Asymmetric Interlaced PCR (TAIL-PCR) identified the position of the T-DNA inserts in four single insertion lines with GFP expressed predominantly in the guard cell. We are analysing the genes and DNA sequences flanking the T-DNA inserts in these four lines. To determine whether the flanking genes are expressed in the guard cell we are using two complementary approaches, RT-PCR of guard cell RNA and promoter-reporter fusions. We are also constructing reporter gene fusions with the DNA sequences immediately upstream of the T-DNA inserts in order to identify potential enhancer/promoter elements that may be driving GFP expression.

### P8.3—Functional analysis of snakeskin, a cell separation mutant in *Arabidopsis thaliana*

C.M. Eland, K. Pyke, A. Marchant and M.J. Bennett, Plant Science, University of Nottingham

Understanding the factors that influence the cell wall properties of plants is of major importance for agriculture and food processing. We have identified an EN transposon-induced mutant known as *snakeskin* (*sk*s) in which the epidermal cell-cell linkages appear to be significantly perturbed.

In *snakeskin* the epidermal cells of the leaves, cotyledons and root 'peel' apart from one another and from the underlying mesophyll cells. As a result of the breakdown of epidermal cell-cell linkages in *sk*s there are changes in epidermal cell architecture and distribution. These include alterations in pavement epidermal cell shape, stomatal distribution and trichome morphology. We will describe the characterisation of the *snakeskin* mutant using a wide range of techniques including reporter genes, cryo-scanning electron microscopy, FT-IR, immunogold labelling and mapping of the *sk*s mutation using SSLP markers.

### P8.4—Designing plant leaves: homeoboxes and beyond

M. Tsantis (University of Oxford)

The shoot apical meristem (SAM) is an indeterminate structure that gives rise to the aerial parts of higher plants. Leaves arise from the differentiation of cells at

the flanks of the SAM. Current evidence suggests that the precise regulation of KNOTTED1-like homeobox (KNOX) transcription factors is central to the acquisition of leaf vs. meristem identity in a wide spectrum of plant species. Factors required to repress KNOX gene expression in leaves have recently been identified. Less is known regarding downstream targets of KNOX function. Recent evidence, however, has suggested that growth regulators may mediate KNOX activity in a variety of plant species.

We have shown that reduced activity of the gibberellin (GA) growth regulator pathway promotes meristematic activity, both in the natural context of KNOX function in the SAM and upon ectopic KNOX expression in *Arabidopsis* leaves. We also have shown that constitutive signaling through the GA pathway is detrimental to the maintenance of meristem indeterminacy. We further have provided evidence that one of the functions of the KNOX protein SHOOTMERISTEMLESS (STM) is to exclude transcription of the GA biosynthetic gene AtGA20ox1 from the SAM. A similar interaction between KNOX proteins and GA biosynthetic gene expression occurs in the tomato leaf and this interaction may contribute to regulation of leaf shape.

Thus transfer of the KNOX/GA regulatory module from the meristem to the leaf may have contributed to the generation of the diverse leaf morphologies observed in higher plants.

### **P8.5—The role of the POLARIS gene in meristem function in *Arabidopsis***

P. Chilley and K. Lindsey, University of Durham, UK

To identify novel genes that regulate root growth and development, a strategy of promoter trapping was carried out on *Arabidopsis thaliana*. One such gene identified in this screen is *POLARIS (PLS)*, *PLS* has been cloned and encodes a predicted polypeptide of 36 amino acid residues. *PLS-GUS* fusion activity is first detectable during embryogenesis, from the early heart stage when the root meristem is being constructed and predominately in the seedling root tip with low expression in the aerial parts. The *pls* mutant exhibits a semi-dominant short root phenotype and reduced leaf vascularization. Studies have demonstrated that *PLS* is expressed not as a feature of cell differentiation per se during root tip construction, but in response to cell position that is independent of cell type at the root tip. A model has therefore been proposed in which *PLS* represents a component of a signalling pathway that defines positional information in the embryonic root.

We are interested in both the role of the *PLS* gene and the nature of the signals that are required for the observed spatially restricted pattern of *PLS* expression which may define at least some aspects of polarity in the *Arabidopsis* root. Evidence is presented that *PLS* is

required for correct auxin-cytokinin signalling to modulate root growth and vascular patterning.

### **P8.6—Transcripts of Vp-1 homeologues are misspliced in modern wheat and ancestral species**

M. Wilkinson, J. <sup>1</sup>Flintham, M. <sup>1</sup>Gale, J. Lenton, M. Holdsworth, (Rothamsted Research, <sup>1</sup> John Innes Centre)

Hexaploid bread wheat (*Triticum aestivum*) caryopses are characterised by relatively weak embryo dormancy and display Pre-Harvest Sprouting (PHS) under cool moist conditions. The phenotype of sprouted wheat is very similar to that of the maize seed-specific mutation *viviparous 1 (vp1)* and to *abi3* in *Arabidopsis*.

VP1 has shown to be a transcription factor that functions both in the activation of genes involved in the maturation program and in the repression of genes involved in germination. Our analysis of the three wheat *Vp-1* genes has shown that each gene produces a set of cytoplasmic mRNAs, the majority of which are incorrectly spliced, containing unedited intron sequences and deletion of coding region. These transcripts do not have the capacity to encode full-length proteins.

The analysis of these *Vp-1* transcripts has been extended to look at their structure in the presumed progenitors of wheat that may allow us to determine when alterations in transcript structures evolved.

‘Gene therapy’ approaches are being used to replace endogenous VP1 activity with *Avena fatua* VP1. Initial results have shown that the presence of the *A/VP1* gene has a positive effect in the ‘sense’ transgenic lines compared with the control and the ‘anti-sense’ line. The germination index of embryos from the sense lines is significantly lower than embryos from the control and anti-sense line on both distilled water and various concentrations of the plant hormone ABA. These initial results will be presented.

### **P8.7—Cereal Functional Genomics**

M. Grimmer, I. Wilson, G. Barker, J. Coghill, K. Edwards, University of Bristol; R. Beswick, Hammersmith Hospital; S. Shepherd, University of Reading; D. Edwards, La Trobe University, Australia

The Bristol Functional Genomics group has developed a range of tools to study cereal transcriptomes. The EU Framework V ZEASTAR programme focuses on maize grain development. ESTs have been derived from specific kernel tissues during grain development, and used to construct a unigene set of ~7500 clones. This will be used initially to provide a comprehensive study of gene expression during grain development in the European model maize line F2. This transcriptome blueprint

is being paralleled by biochemical and proteomic analysis. The BBSRC-funded Investigating Gene Function (IGF) project focuses on wheat grain development and also the effects of various environmental stresses. To do this a unigene set of ~10 000 ESTs has been constructed. A maize transposon tagging grid was set up, and the flanking sequences have been microarrayed for screening with genes of interest. As part of the bioinformatics resources for the IGF program, AutoSNP software has been developed for mining SNPs from redundant EST sequence alignments. This programme has been applied to EST data from public databases, and has produced over 3000 candidate SNP loci for both maize and wheat. Further information on these projects can be found at the following URL: <http://www.cerealsdb.uk.net>. This website can also be used to search the wheat EST database, the SNP database and a transposon flanking sequence database. In my talk I will provide an update on the various programmes and potential future developments.

### **P8.8—Opportunities to find candidate genes for QTLs of small effect from genome sequence information: Reflections on QTLs for root growth and drought avoidance in rice**

A.H. Price, F.S. Khowaja and A. McIntosh, Biology, University of Aberdeen

Modifying the root morphology of rice should allow improvements in nutrient and water capture in marginal soils. Quantitative trait loci (QTL) for root morphological traits have been identified in several mapping populations in rice and have been applied to marker assisted selection (MAS) projects. In all cases, QTL are small ( $r^2$  between 5 and 20%) and are generally environmentally sensitive. Identifying the gene responsible for QTL would greatly aid understanding of the genetic control of root growth, aid MAS and facilitate transgenic approaches to root modification. Genome sequence information should facilitate map-based gene identification. However, small, environmentally unstable QTL are not amenable to gene tagging. This presentation considers the prospects for using original QTL mapping data to identify candidate genes without the step of gene tagging. Issues concerning the types of genes to consider, QTL precision, allele diversity, gene families, gene annotation, predicting gene function and proof of function are presented. Using data from a Bala x Azucena F6 population, some examples are given including both expansins and that of the *sd1* locus where the gene has recently been identified. It is concluded that significant 'risks' exist that will limit the success of identifying a gene for each QTL. However, there is reason to suppose that it might be successful and cost effective for some

QTL, particularly if multiple data sets can be used to improve QTL precision.

### **P8.9—The Regulation of Seed oil breakdown in *Arabidopsis***

S. Penfield, I. Graham; University of York

Oilseeds such as *Arabidopsis* store carbon in the form of triacylglycerol (TAG) and this is mobilised to fuel seedling establishment and post-germinative growth. Fatty acids from TAG are catabolised by  $\beta$ -oxidation, producing acetyl-CoA that is converted to sucrose through the glyoxylate cycle and gluconeogenesis. The abundance of key enzymes in lipid mobilisation peaks at 2 days post germination and northern blots demonstrate that this expression pattern is regulated predominantly at the transcript level. Furthermore, promoter::reporter fusions for PED1 (thiolase), malate synthase, isocitrate lyase and phosphoenolpyruvate carboxykinase show that the rate of transcription is the pivotal regulatory step. To further investigate the factors required for this regulation, a transgenic line expressing the luciferase reporter gene under the control of the malate synthase promoter was mutagenised. 14 mutants designated *REGULATOR OF THE GLYOXYLATE CYCLE* (*RGC*) have been identified that show an increase in luciferase activity after germination. A preliminary characterisation of these is presented.

### **P8.10—Dissecting polar membrane transport in plants**

M.J. Bennett, G. Lycett and R. Swarup, University of Nottingham; Ian Moore, University of Oxford.

Localisation of the AUX and PIN families of auxin transporters has recently demonstrated for the first time that root cell types can maintain a steady state polar distribution of membrane proteins (1, 2). The presumptive auxin efflux carrier PIN1 is localised to the lower PM face of root stele cells. In contrast, the presumptive auxin influx carrier AUX1 is asymmetrically localised to the upper PM face of protophloem cells but exhibits a non-polar distribution in the PM of lateral root cap cells (1). Simultaneous localisation of AUX1 and PIN1 in *Arabidopsis* roots (1) has shown that these two PM proteins adopt distinct polar distributions in a single protophloem cell. Selective apical and basal targeting of PIN1 and AUX1 by the membrane trafficking pathways would be an attractive way to explain the polarised distributions of these proteins. We and others have recently identified *Arabidopsis* mutations that disrupt targeting of PIN1 (3) or AUX1 proteins. Polar targeting of AUX1 to the upper PM face of protophloem cells is selectively disrupted in the *Arabidopsis* *axr4* mutant, whilst PIN1 is localised normally. Our *axr4* results represent the first

genetic evidence in plants that targeting of apical and basal PM markers is mediated by distinct molecular mechanisms.

- (1) Swarup et al (2001) *Genes & Development*, 15, 2648–2653
- (2) Galweiler et al (1998) *Science* 282: 2226–2230
- (3) Steinmann et al (1999) *Science*, 286, 316–318

### **P8.11—Molecular characterisation of the GA 20-oxidase-1 promoter from *Arabidopsis*: Identification of cis-elements that mediate control of gene expression by gibberellins**

Omar Ruíz; Jeremy Coles; Peter Hedden & Andy Phillips (Rothamsted Research)

Gibberellins are a class of plant hormones that are involved in a wide range of developmental responses. The processes regulated by GAs are limited by the concentration of biologically active GAs, and therefore the regulation of GA biosynthesis is an important factor in the control of plant development. Later part of GAs biosynthetic pathway is catalysed by the enzyme GA20-oxidase. *GA20ox* genes are down-regulated by bioactive GAs in a feedback mechanism that maintains the concentration of GAs within plant tissue. A major part of the feedback control over GA20-oxidase gene expression operates at the transcriptional level. *Arabidopsis* transgenic plants containing a translationally construct *AtGA20ox1* promoter-*gus* cassette show a marked up-regulation of the reporter gene when seedlings were treated with paclobutrazol, reflecting the expression pattern of the endogenous *AtGA20ox1* gene and its feedback-regulation by GAs. Analysis of a 5' deletion series of *AtGA20ox1* promoter in transgenic plants demonstrates that the sequence elements within a 379 bp proximal promoter fragment are enough to confer tissue-specific expression and response to GAs. In vivo footprinting of the *AtGA20ox1* promoter reveals differences in the methylation patterns between seedlings and pollen. Differentially methylated G-residues overlap near-palindrome sequences and direct repeat motifs. These motifs are, therefore, prime candidates for regulatory elements that control *AtGA20ox1* gene expression and possibly mediate the feedback response to GAs.

### **P8.12—A transcriptomics approach to studying axillary meristem dormancy in *Arabidopsis thaliana***

Sally Ward and Ottoline Leyser, Biology, University of York

The aerial architecture of flowering plants, such as *Arabidopsis thaliana*, is largely determined by shoot growth

and shoot branching arising from the initiation and growth of axillary meristems. The complexity of this branching appears dependent upon genetic cues but is also highly responsive to the environment. Axillary meristems form in the axils of leaves where they can remain dormant or can activate to produce shoot branches. Understanding of the genetic processes involved in this transition from dormant to active axillary meristems could have important consequences in agriculture, horticulture and forestry.

A transcriptomics approach is being used to study this transition. Initially transcripts are being identified as being differentially expressed in dormant and actively growing axillary buds from microarray studies. This includes a study of mRNA populations from the axillary buds of the auxin over-responding, apically dominant *axr3-1* mutant compared with the axillary buds of the auxin resistant *axr1-12* bushy mutant. After using these studies to identify differentially expressed genes, two types of genes will be selected for further analysis. These will either be developed into potential molecular markers for meristem status or, if they appear to be candidate regulatory genes they will be characterised further and insertional mutants looked for. The datasets will be compared with those from other meristems that undergo phases of dormancy ie seed and cambium and transcripts selected that are specific to some or all stages of dormancy. These will be used to make dormancy microarrays allowing expression profiles to be produced of dormancy-related *Arabidopsis* mutants.

### **P8.13—Identification of guard cell genes using the GAL4-GFP enhancer trap system**

A.J. Baker, S.W. Hou, Department of Plant Sciences, University of Cambridge, UK; R.S. Poethig, University of Pennsylvania, Department of Biology, Plant Science Institute, Philadelphia, Pennsylvania, USA; J. Haseloff and A.A.R. Webb, Department of Plant Sciences, University of Cambridge, UK

Stomatal guard cells have been studied extensively using electrophysiology, Ca<sup>2+</sup> and pH imaging techniques, gas exchange measurements and biochemical analyses. As a result we are now beginning to understand some of the networks of pathways and downstream targets involved in guard cell signal transduction. Our knowledge of the genes involved in both guard cell development and function is much less advanced. We are using the GAL4-GFP enhancer trap system to identify genes involved in guard cell function and/or development. The GAL4-GFP enhancer trap electronic databases were screened for lines expressing GFP in the stomatal guard cells. 22 lines were selected on the basis of stable GFP expression in the guard cells and not more than 3–4 other cell/

tissue types. The T-DNA copy number in each of these guard cell enhancer trap lines was determined using Southern blots. Two lines were identified with single T-DNA insertions and two more single insertion lines were generated by segregation following backcrossing of the enhancer trap line with the wild type background. Thermal Asymmetric Interlaced PCR (TAIL-PCR) was used to identify the position of the T-DNA inserts in the single insert lines. In each of these single insert lines GFP was expressed in the guard cell but the insert positions varied from intergenic to intragenic. We are analysing the expression patterns of the genes flanking the T-DNA inserts and analysing the genomic DNA sequence adjacent to the T-DNA in order to identify potential enhancer/promoter elements.

#### **P8.14—Investigating vascular development using damian and other mutants**

P.J. Perry, N.S. Graham, A. Marchant, M.J. Bennett, Plant Sciences, University of Nottingham

The development of the vascular system has been poorly understood and one of the main theories has been challenged by recent publications (e.g. Koizumi et al, 2000 Development 127,3197–3204). This poster will describe a number of different genetic approaches to study vascular development, these include both forward and reverse genetics.

Enhancer trapping has been used to identify novel expression patterns, and mutations within the vascular system. A range of both temporal and spatial patterns within the vascular system will be described here. One vascular mutant, *damian*, exhibits discontinuous xylem development. This mutant also has a number of other phenotypes will be described.

The POPWOOD project uses reverse genetics to find mutations in the vascular system. Expressed gene sequences from the phloem in poplar were used to identify orthologous genes within *Arabidopsis*; the function of these genes was studied.

Other mutants with vascular abnormalities have been identified using a novel transgene based screen.

#### **P8.15—Differential expression of 14-3-3 genes in *Arabidopsis thaliana***

G. Lingiah, S. Comparot, N. Marinsek and T. Martin, Department of Plant Sciences, University of Cambridge, UK

The eukaryotic 14-3-3 multigene family encodes for proteins involved in the regulation of diverse processes, such as development, cell-cycle control, responses to stress and regulation of metabolic enzymes. Regulation of these processes involves, in most cases, binding of a 14-3-3 homo or hetero dimer to a phosphorylated target

protein. In plants, among other functions, 14-3-3 bind to and are involved in the regulation of a number of key enzymes in primary carbon and nitrogen metabolism e.g. NR, SPS and GS. It is hypothesised that 14-3-3 proteins play a crucial role in co-ordinating plant carbon and nitrogen metabolism. The numerous functions of 14-3-3 proteins, their action as homo and hetero dimers, and the presence of 14-3-3 multigene families lead to the question of 14-3-3 specificity. Specificity could be mediated by spatial, temporal and environmental control of gene expression. Here we present an attempt to address this issue, using semi-quantitative multiplex RT-PCR to analyse the expression of individual members of the *Arabidopsis* 14-3-3 gene family. The data presented show differential regulation of 14-3-3 genes by carbon and nitrogen status as well as by tissue type.

#### **P8.16—Post-transcriptional regulation of the pea *plastocyanin* gene (*PetE*)**

N.J. Brown and J.C. Gray, Department of Plant Sciences, University of Cambridge

Plastocyanin is encoded by the intronless nuclear gene, *PetE*. Expression of pea *PetE* is regulated both by light and by signals from the chloroplast in pea and in transgenic tobacco and *Arabidopsis* plants. The regulation has been shown to operate post-transcriptionally, requiring the correct 5' terminus of the transcript and elements within the plastocyanin-coding region (Helliwell et al., 1997; Sullivan and Gray, 2002). The mechanism by which light and plastid signals influence the stability of the transcripts is being investigated. *PetE* constructs which examine the roles of the 5' terminus of the transcript and of the coding region in this process have been generated. Transgenic tobacco and *Arabidopsis* plants containing these constructs have been produced and RNA-gel-blot analysis and luciferase assays have been carried out on the seedlings to examine the effects of light and plastid inhibitors, such as norflurazon and lincomycin, on *PetE* expression. Transgenic tobacco plants containing *PetE* constructs with premature stop codons introduced in the coding region, suggest that translation is required for both light and plastid-controlled regulation to operate.

Helliwell, C.A., Webster, C.I. and Gray, J.C. (1997) Light-regulated expression of the pea plastocyanin gene is mediated by elements within the transcribed region of the gene. *Plant J.* **12**: 499–506.

Sullivan, J.A. and Gray, J.C. (2002). Multiple plastid signals regulate the expression of the pea plastocyanin gene in pea and transgenic tobacco plants. *Plant J.* **32**: 763–774.

#### **P8.17—**

Abstract withdrawn

**P8.18—Molecular characterisation of the GA 20-oxidase-1 promoter from *Arabidopsis*: Identification of cis-elements that mediate control of gene expression by gibberellins**

Omar Ruíz; Jeremy Coles; Peter Hedden & Andy Phillips (Rothamsted Research)

Gibberellins are a class of plant hormones that are involved in a wide range of developmental responses. The processes regulated by GAs are limited by the concentration of biologically active GAs, and therefore the regulation of GA biosynthesis is an important factor in the control of plant development. Later part of GAs biosynthetic pathway is catalysed by the enzyme GA20-oxidase. *GA20ox* genes are down-regulated by bioactive GAs in a feedback mechanism that maintains the concentration of GAs within plant tissue. A major part of

the feedback control over GA20-oxidase gene expression operates at the transcriptional level. *Arabidopsis* transgenic plants containing a translationally construct *AtGA20ox1* promoter-*gus* cassette show a marked up-regulation of the reporter gene when seedlings were treated with paclobutrazol, reflecting the expression pattern of the endogenous *AtGA20ox1* gene and its feedback-regulation by GAs. Analysis of a 5' deletion series of *AtGA20ox1* promoter in transgenic plants demonstrates that the sequence elements within a 379 bp proximal promoter fragment are enough to confer tissue-specific expression and response to GAs. In vivo footprinting of the *AtGA20ox1* promoter reveals differences in the methylation patterns between seedlings and pollen. Differentially methylated G-residues overlap near-palindrome sequences and direct repeat motifs. These motifs are, therefore, prime candidates for regulatory elements that control *AtGA20ox1* gene expression and possibly mediate the feedback response to GAs.