

## C5/P3–SIGNALLING CROSS–TALK

Organised by J.E. Taylor and M.R. McAinsh for the Plant Development and Cell Signalling Groups and Sponsored by the Journal of Experimental Botany

### C5/P3.1–Straight-talk about cross-talk in plant–herbivore interactions

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Microarray technology has given plant biologists the ability to simultaneously monitor changes in the expression of hundreds of genes, and yet, to date this technology has not been used to characterize the output of the network of interwoven, cross-talking cascades that mediate environmentally realistic responses: the imprint made by these responses on a plant's transcriptome. We use a cDNA microarray, consisting of 240 herbivory-related genes of the model ecological expression system, *Nicotiana attenuata*, and with a principle components analysis (PCA), characterize transcriptional imprints in response to single, sequential, or simultaneous attack by the plant's two main herbivores, the chewing larvae of *Manduca sexta* and the cell-content feeding nymphs and adults of *Tupiocoris notatus* which both have profoundly different fitness consequences for the plant. The PCA identified distinctly different imprints left by attack from the two individual species and the attack from both combined. When attacked sequentially, the *Manduca* imprint was more ephemeral than that from a mirid attack, which is consistent with our findings of a mirid-mediated vaccination of *N. attenuata* plants observed in natural populations. The dissection of the transcriptional imprints, revealed a startling result: only 10 genes were solely species-specific regulated. All other regulated genes (136) revealed ubiquitous or context-dependent regulation, being either erased or stimulated by attack order. For researchers interested in ecological performance, understanding how context-dependent expression arises from simple combinations of wound and herbivore-specific elicitors should be a major goal.

### C5/P3.2–Ecological consequences of signaling crosstalk in tomato plants

J.S. Thaler, Botany, University of Toronto

Signaling cross-talk can result in trade-offs in plant responses to multiple stresses. I will examine the ecological consequences of trade-offs in the expression of the jasmonate and salicylate responses for plant resistance to insects and pathogens, and also address roles for ABA in plant resistance. I will first address what set of organisms are affected by each response. To what

degree do the jasmonate- and salicylate-responses individually contribute resistance to a diverse array (14 species) of insects and pathogens with varying feeding styles. Experiments demonstrate that the jasmonate-response provides resistance to almost all insects and many pathogens. On the other hand, the salicylate-response protects plants against almost all pathogens, but none of the insects studied. Second, how does cross-talk influence the resistance provided by each pathway? If expression of the jasmonate response is attenuated by expression of the salicylate response, how is jasmonate-mediated *resistance* affected. Attenuation of the jasmonate-response by the salicylate-response resulted in attenuation of resistance in only two of the five insect species studied. Finally, are there ecological scenarios that would favor the evolution of signaling cross-talk? What is the correlation in space and time between attack by insects and pathogens? Are both parasites equally harmful to the plant and is there genetic variation for the signaling cross-talk? Answering these questions is important for understanding whether cross-talk is due to genetic/physiological constraints or whether it represents an adaptation for coping with hyper-variable environments.

### C5/P3.3–Crosstalk between plant responses to pathogens and herbivores: A view from the outside in

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The conventional definition of crosstalk refers to the interaction of signalling pathways at the cellular level. In an ecological context crosstalk could refer to the physiological outcome of a plant's response to attack by a range of organisms, either simultaneously or in sequence. This paper will review the research detailing the physiological response to attack by multiple enemies; this will include details of the pathways known to be induced by different pests and pathogens, and the evidence to support the potential for crosstalk at the cellular level. We will identify the type of molecular questions and approaches that might be used to gain more insight into the mechanisms by which plants respond to attack by multiple enemies.

### C5/P3.4—Signals for local and systemic responses of plants to pathogen attack

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In systemic acquired resistance (SAR), inoculation of one leaf of a plant, leading to local hypersensitive resistance, is followed by generation of a systemic signal that transduces the resistance response to other parts of the plant such that attempted secondary infections are rapidly and effectively blocked. Screening *Agrobacterium* T-DNA tagged lines of *Arabidopsis thaliana* for mutants specifically compromised in SAR led to the identification of *defective in induced resistance 1-1* (*dir1-1*) that exhibits wild-type local resistance to avirulent and virulent *Pseudomonas syringae*, but lack of SAR and *PR* gene expression in uninoculated distant leaves. *DIR1* encodes a putative apoplastic lipid transfer protein that might interact with a lipid-derived molecule to promote long distance signaling during SAR. We have also employed T-DNA activation tagging to uncover genes involved in enhanced resistance to bacterial infection. *Cdr1-D* (constitutive disease resistance 1) showed enhanced resistance to *P. syringae* associated with constitutive activation of *PR* genes, NADPH oxidase, salicylic acid (SA) levels, spontaneous microbursts of H<sub>2</sub>O<sub>2</sub> and microscopic lesions. SA is necessary for *cdr1-D* mediated induction of defense responses. *CDR1* encodes an extra-cellular aspartic protease that might be involved in the generation of a polypeptide signal (elicitor) that could be a mobile SAR signal.

The biosynthetic origin of SA during plant defense is not fully understood. We have examined the role of the phenylpropanoid pathway in provision of SA by observing the effects of phenylalanine ammonia-lyase over-expression on the response of tobacco to viral and fungal pathogens.

### C5/P3.5—Vitamin C contents modulate genes involved in plant defense and development

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Vitamin C (Ascorbic acid) is a major antioxidant buffer in plants. Ascorbate deficiency in the *Arabidopsis thaliana vtc1* mutant causes slow growth and late flowering. The mutant has only 30% of the leaf ascorbate found in the wild type (Col0) but H<sub>2</sub>O<sub>2</sub> contents and the redox states of the ascorbate and glutathione pools are similar in both mutant and wild type. *Vtc1* leaves show constitutive induction of a number of pathogenesis-related

(PR) transcripts. These include *PR-1*, *PR-2*, a thaumatin-like *PR-5*, a  $\beta$ -1,3 glucanase and an endochitinase. However, transcripts encoding phenylalanine ammonia lyase were similar in *vtc1* and Col0. Hence, low ascorbate in *vtc1* is associated with the induction of PR proteins through signalling pathways that appear to be independent of active oxygen species. When challenged with a virulent race of *Pseudomonas syringae* pv tomato, pathogen proliferation was reduced by almost 15–20 fold at 5 days post-infection in *vtc1* compared to the wild type. Transcript changes indicate that growth and development are constrained by modulation of the balance between abscisic acid (ABA) and gibberellic acid (GA) signaling. In agreement, ABA is significantly higher in *vtc1* than the wild type and spraying with GA rescues the growth phenotype. The ascorbate content of *vtc1* leaves was increased 14 fold after supplying 10 mM ascorbate, without a concomitant change in redox state. Following this treatment *PR-1* transcripts were repressed, whereas *PR-5* and *PR-2* transcripts were not significantly changed. *PR-1*, *PR-2* and *PR-5* transcripts do not therefore appear to be co-ordinately expressed in the *vtc1* mutant. Transcripts encoding dehydroascorbate reductase, pathogenesis-related protein1 and a peroxiredoxin were decreased while those encoding salicylate induction-deficient protein1, CuZn superoxide dismutase (SOD), Fe-SOD, metallothionein and glutathione transferases were increased. High ascorbate also caused decreases in mRNAs encoding chloroplast enzymes such as fructose-1,6-bisphosphatase and sedoheptulose-1,7-bisphosphatase that are activated by reduced thioredoxin. In contrast, others such as glucose 6-phosphate dehydrogenase, whose activity is inactivated by reduced thioredoxin, were repressed. These data show that ascorbate is involved in the metabolite 'cross-talk' between different parts of the defense network. Ascorbate abundance provides information on redox buffering capacity that co-ordinates redox processes associated with the regulation of photosynthesis and plant defence.

### C5/P3.6—CDPKs, molecular switches between biotic and abiotic stress responses

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Calcium-dependent but calmodulin-independent protein kinases (CDPKs) are a family of protein kinases that may function as major primary sensors for calcium signals in plants.

Recently, we showed that a tobacco CDPK, *NtCDPK2*, is transcriptionally and post-translationally activated after race-specific elicitation and hypo-osmotic stress. Furthermore, *NtCDPK2* activation is required for plant

defence responses (Romeis et al., 2001). To identify CDPK-controlled signalling pathways we ectopically expressed a truncated version of *NtCDPK2* in *Nicotiana benthamiana*. This truncated version lacked both regulatory junction and calmodulin-like domain. Upon ectopic expression of this truncated *NtCDPK2*, plants responded to an abiotic stress stimulus (infiltration of water) with an inappropriate activation of biotic defence responses such as rapid accumulation of ROS, induction of basic pathogenesis-related genes, and hypersensitive response (HR)-like cell death. In addition, *NtCDPK2* variant expression resulted in increased levels of jasmonic acid, 12-oxo-phytodienoic acid and ethylene, and coincided with the inhibition of abiotic and biotic stress-induced mitogen-activated protein (MAP) kinase activation. This inhibition was compromised in the presence of silver ions, which block ethylene perception.

Our data identify *NtCDPK2* as a mediator between abiotic and biotic stress signalling activating JA/ethylene-dependent cellular responses. We also demonstrate for the first time cross talk between CDPK and MAPK pathways, and ethylene represents the molecular link between both signalling branches.

Romeis, T., Ludwig, A.A., Martin, R., and Jones, J.D.G. (2001). Calcium-dependent protein kinases play an essential role in a plant defence response. *EMBO J.* 20: 5556–5567.

### **C5/P3.7—Flagellin, a bacterial protein, functions as a ‘PAMP’ in plants**

Thomas Boller, Botanisches Institut, Universität Basel, Switzerland

After decades of neglect, ‘innate immunity’ in animals has recently moved to center stage, with the discovery that a first line of defense against infection is brought about by Toll-like receptors via the recognition of ‘pathogen-associated molecular patterns’ (PAMPs). Conceptually, the classic model of the plants’ defense against infection has a very similar basis: Microbial ‘elicitors’ have long been known to activate the first line of defense in plants. We have recently discovered that plants have a highly specific and sensitive perception system for bacterial flagellin and the peptide flg22, representing the most conserved domain of flagellin. The *FLS2* gene, which is involved in flagellin perception, encodes a receptor kinase with an extracellular domain resembling Toll-like receptors in animals. In fact, one of the latter receptors, TLR5, has now been reported to mediate flagellin perception as well. Thus, the plants’ perception systems for elicitors resemble the recognition systems for PAMPs in the animal innate immunity response. We used microarray chips of Affymetrix to examine changes in gene expression after flg22 stimulation. An initial analysis of the expression pattern in seedlings challenged for 30 min with flg22 indicates a

massive change in gene expression. Interestingly, among the genes induced are many encoding transcription factors and other components of signalling cascades, and a considerable number of resistance genes, indicating that the flg22 stimulus may increase the ‘awareness’ of the plant cell for more specific stimuli as the ones recognized by the resistance genes.

### **C5/P3.8—A MAP kinase is associated with the actin cytoskeleton and regulates root hair formation and tip-growth**

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Mitogen-activated protein kinases (MAPKs) are versatile transducers of plant signalling (Jonak et al. (2002) *Curr. Op. Plant Biol.* 5, 415–424). MAPKs phosphorylate and thereby regulate diverse targets including transcription factors, cytoskeletal components and other protein kinases. In this way, MAPKs can participate in transcriptional regulation of genes and post-translational modification of gene products including those which are directly involved in plant development and cytoskeletal organization. The stress-induced Medicago MAPK SIMK is activated by osmotic stress and elicitors. Expression studies in roots revealed nuclear localisation of SIMK in epidermal cells but association with actin filaments in growing root hairs. Treatment of root hairs with actin drugs or a MAPKK inhibitor affected the actin cytoskeleton and stopped root hair growth. Expression of constitutively active SIMK resulted in longer root hairs (Samaj et al. (2002) *EMBO J.* 21, 3296–3306). These data show that SIMK and actin play important roles in root hair tip growth and provide the first link for a regulation of the actin cytoskeleton by MAPKs in plants.

### **C5/P3.9—Crosstalk in guard cell signalling**

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The aperture of the stomatal pore is regulated by a wide range of important environmental stimuli. Recent work has identified many new components in guard cell signalling with the ABA signal transduction pathway being the most intensively investigated. Changes in stomatal aperture are brought about by co-ordinated changes in guard cell turgor, cytoskeletal organization, membrane trafficking and possibly gene expression. This presentation will describe the role of the calcium ion in guard cell signalling.

### C5/P3.10—ABA, hydrogen peroxide and nitric oxide signalling cross-talk in stomatal guard cells

R. Desikan, M.-K. Cheung, J.T. Hancock and S.J. Neill, Centre for Research in Plant Science, University of the West of England

Water deficit stress results in increased biosynthesis of the phytohormone abscisic acid (ABA). ABA induces stomatal closure, via activation of a complex network of signalling cascades resulting in loss of turgor and shrinkage of guard cells surrounding stomatal pores. Recent research has highlighted the requirement for novel signalling intermediates such as hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), and in our laboratory, nitric oxide (NO). Guard cells of *Pisum sativum* and *Arabidopsis thaliana* generate NO and H<sub>2</sub>O<sub>2</sub> following ABA treatment, and both H<sub>2</sub>O<sub>2</sub> and NO synthesis are required for full stomatal closure. Treatment with exogenous NO also induces stomatal closure, and ABA and NO responses involve the synthesis and action of the second messengers cyclic GMP and cyclic ADP ribose. Pharmacological and biochemical data indicate that the source of NO in *P. sativum* epidermal peels may be via a potential nitric oxide synthase (NOS)-like enzyme, whereas in *Arabidopsis* nitrate reductase (NR) is indicated. Biochemical and molecular data point to H<sub>2</sub>O<sub>2</sub> synthesis via NADPH oxidase. Some insights into the interaction between H<sub>2</sub>O<sub>2</sub> and NO during the ABA signal transduction process will be discussed.

### C5/P3.11—Crosstalk in cold and drought signalling and in pathways using active oxygen species

M.R. Knight, Plant Sciences, University of Oxford

Our lab has been working for some years on abiotic stress signalling pathways. We have tended to think of these exclusively in terms of independent linear pathways, leading from specific stresses to specific end responses, not considering interaction between pathways. Recently, 2 aspects of our work have forced us to consider crosstalk. Firstly in the cold and drought signalling pathways, the *sfr6* mutant of *Arabidopsis*, appears to link cold and drought (and possibly other signals) far downstream at the point of transcriptional activation of stress genes. Secondly, our work on hydrogen peroxide signalling has identified a protein kinase, OX1, which seems to integrate the reactive oxygen signals from several different stresses e.g. pathogens and wounding as well as developmental responses e.g. root hair formation and flowering. I will discuss these findings and their implications.

### C5/P3.12—Expression profiling using *Arabidopsis* full-length cDNAs under abiotic stress conditions

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Full-length cDNAs are essential for the correct annotation of genomic sequences and for the functional analysis of genes and their products. Using the biotinylated CAP trapper method, we constructed full-length cDNA libraries from *Arabidopsis* plants<sup>1,2</sup> and isolated 155,144 RIKEN *Arabidopsis* full-length (RAFL) cDNA clones. The 3'-end ESTs of 155,144 RAFL cDNAs were clustered into 14,668 non-redundant cDNA groups, about 60% of predicted genes<sup>3</sup>.

We prepared a new version of full-length cDNA microarray containing ca. 7000 independent full-length cDNA groups to analyze the time course of gene expression under abiotic stress conditions<sup>4,5,6</sup>. The transcripts of 53, 277 and 194 genes increased after cold, drought and high-salinity treatments, respectively, more than 5-fold compared with the control genes. Among them, we found 40 (corresponding to ca. 11% of all stress-inducible genes identified) transcription factor genes, suggesting that various transcriptional regulatory mechanisms function in the drought-, cold- or high-salinity-stress signal transduction pathways. Recently, we also identified 154 rehydration-inducible genes. Detailed characterization of the rehydration-inducible genes will be presented.

1) Seki et al. (1998) Plant J. 15:707–720. 2) Seki et al. (2001) Plant Physiol. Biochem. 39: 211–220. 3) Seki et al. (2002) Science 296:141–145. 4) Seki et al. (2001) Plant Cell 13:61–72. 5) Seki et al. (2002) Plant J. 31:279–292. 6) Seki et al. (2002) Funct. Integr. Genomics 2:282–291.

### C5/P3.13—Genetic analysis of signalling pathways for salt, drought and cold regulated gene expression

S. Kanrar and J.-K. Zhu (Arizona, USA)

Abstract not supplied

### **C5/P3.14—Maintenance of root and shoot growth by ABA: the role of ethylene suppression**

R.E. Sharp, M.E. LeNoble and I.-J. Cho, University of Missouri-Columbia, USA

ABA is generally considered as an inhibitor of growth. This view has arisen largely from studies of effects of exogenous ABA. However, the use of ABA-deficient mutants to manipulate endogenous ABA levels and methods to avoid variation in plant water status due to low ABA has demonstrated that (a) the normal ABA levels in well-watered plants are required to maintain shoot growth in tomato and *Arabidopsis*, and (b) the accumulation of ABA under water stress is required for root growth maintenance in maize seedlings. In all cases, the action of ABA involves suppression of ethylene production. To assess the extent to which ABA maintains shoot growth by ethylene suppression, the growth of ABA-deficient (*aba2-1*) and ethylene-insensitive (*etr1-1*) single- and double-mutants was examined in well-watered *Arabidopsis*. The results confirm that ethylene is an important cause of the inhibition of shoot growth in *aba2-1*, but indicate that ABA has another function in maintaining shoot growth in addition to ethylene suppression. Taken together, our studies indicate that water-stressed compared to well-watered plants require increased ABA to prevent ethylene-induced growth inhibition. This difference may be related to a role of ABA in up-regulating antioxidant enzyme activities during water stress. Levels of reactive oxygen species (ROS) are considerably increased in the growth zone of ABA-deficient (*vp14* mutant) compared to wild-type maize roots under water-stressed but not under well-watered conditions. The possibility that increased ROS levels are causally related to increased ethylene production and growth inhibition of ABA-deficient roots under water stress is under investigation.

### **C5/P3.15—Interactions between development and hormone signaling in *Arabidopsis***

P. McCourt and B. Chow, University of Toronto, Canada

Many of our notions about how plant hormones transduce a signal into a cellular response come from studies of hormone response mutants identified in *Arabidopsis thaliana*. Genetic inferences taken from *Arabidopsis* mutants that hormone signaling pathways are linear have never been reconciled easily with physiological studies of hormone action. The application of a single hormone often affects many different plant processes and multiple hormones can influence the same process. In this presentation I will touch upon how more careful phenotypic

analysis of known hormone response mutants in *Arabidopsis* indicates these mutations affect multiple hormone-regulated responses. Hence, genetic analysis of hormone action appears to be more complex than originally suggested. Related to this, I will also present results showing that known developmental regulators can also affect hormone biosynthesis and action.

### **C5/P3.16—Sugar and phytohormone response pathways: navigating a signaling network**

S.I. Gibson, Plant Biology, University of Minnesota

The levels of soluble sugars, such as glucose and sucrose, affect a diverse array of metabolic and developmental processes. Many of these processes are also affected by the levels of one or more phytohormones. For example, seed germination is negatively regulated by soluble sugars and abscisic acid and is positively regulated by ethylene and gibberellins. Expression of many genes is also regulated by both sugars and phytohormones. However, the mechanisms by which sugar and phytohormone response pathways 'interact' in control of these processes remain poorly understood. Recent results from several labs are aiding in the development of models to describe these interactions. For example, sugar-insensitive mutants of *Arabidopsis* that are resistant to the inhibitory effects of high concentrations of exogenous sugars on seed germination and early seedling development have been identified and characterized. Several of these mutants also exhibit defects in phytohormone response or metabolism. For instance, some sugar-insensitive mutants are also resistant to the inhibitory effects of abscisic acid and/or paclobutrazol (an inhibitor of gibberellin biosynthesis) on seed germination. Interestingly, not all sugar-insensitive mutants are also abscisic acid or paclobutrazol insensitive and not all abscisic acid or paclobutrazol insensitive mutants are also sugar insensitive. These results indicate that the connections between sugar and phytohormone response pathways, whether direct or indirect, exhibit a significant degree of specificity. Insight into interactions between sugar and phytohormone response pathways is also being provided by DNA chip experiments that are identifying the subsets of genes regulated in response to different sugar and phytohormone signals.

### **P3/C5.17—Signal integration during photomorphogenesis in *Arabidopsis* seedlings**

J.L. Nemhauser, Salk Institute and J. Chory Salk Institute and Howard Hughes Medical Institute, La Jolla, CA

Hormones are at the heart of all plant growth and development; yet, the link between extrinsic or intrinsic cues

and specific outputs is largely unknown. Auxin and brassinosteroids (BRs) are required for a wide variety of vital growth processes in plants, including the light response of emergent seedlings. In a wide range of tissues and species, auxins and BRs work synergistically to promote cell elongation. We are using a combination of physiological, genetic, and molecular tools to characterize BR-auxin synergism in *Arabidopsis*. By increasing the response output for a given hormone treatment, BR-auxin synergism can be used to design sensitized screens to identify new components required for the effects of each individual hormone. We have performed one such screen and identified several mutants, termed *auxin-brassinosteroid enhanced* (*abe*), which show increased sensitivity to both hormones in a hypocotyl elongation assay. In addition, we are using global gene expression analysis to further elucidate the overlapping response of auxin and BRs, as well as to identify factors regulated in response to both hormones. Ultimately, analysis of the interaction between these two hormones will provide an entry point for dissecting the diverse molecular mechanisms underlying signal integration in complex processes, such as seedling photomorphogenesis.

### **C5/P3.18—Light signals, phytochromes and crosstalk with other environmental cues**

G.C. Whitelam, P.J. Ingles, M.G. Salter and K.A. Franklin, Department of Biology, University of Leicester, UK

Light signals regulate all aspects of plant growth and development, and so play a crucial role in determining the architecture of plants. Red- and far-red light signals are perceived by the phytochrome family of photoreceptors. Higher plants possess multiple phytochromes and in *Arabidopsis thaliana* the phytochrome family comprises five members (phyA to phyE), the apoproteins of which are encoded by five discrete genes, *PHYA-PHYE*. Genetic approaches are revealing the complexity of phytochrome actions and interactions. Different phytochromes can have different or overlapping functions, they can act redundantly and can interact with one another and with other photoreceptors or other regulatory systems, including other environmental cues, such as gravity and temperature. Through the identification of phytochrome-null mutants, we are now able to determine the functions and interactions of every single member of the phytochrome family. In light-grown plants, phytochromes B, D and E act redundantly to perceive the low red:far-red ratio light signals reflected from nearby vegetation and to regulate plant architecture and flowering time: the so-called shade avoidance syndrome. The most rapid facet the shade avoidance response, increased elongation growth, is gated by the circadian clock and involves rapid regulation of gene expression.

### **C5/P3.19—Dissecting the plant circadian clock**

A.J. Millar, A. Hall, P.E. Brown, Biological Sciences; J. Locke, M.S. Turner, Physics; B. Shulgin and D. A. Rand, Mathematics, University of Warwick

About 20 interacting genes have been shown to function in the core of the circadian system and the photoperiodic sensor. In addition, at least 7 photoreceptors initiate light signalling to reset the clock, synchronising biological time with the day/night cycle. The clock has a complex interaction with light signalling pathways, including rhythmic regulation of photoreceptor gene expression, subcellular localisation and biological function. We have identified new components of the plant circadian clock, including a small protein, ELF4, which functions at the end of the day. Increasingly detailed results from molecular genetics do not necessarily lead to greater understanding of such a regulatory network and mathematical modelling can provide a valuable, complementary approach. We are developing 'complete' models for the plant clock and photoperiod sensor, which incorporate the molecular components in a realistic manner. In particular, we have established a novel analytical method to assess the contribution of each component of the model (RNA or protein) at each phase of the cycle, which helps to understand their functions even in very complex models. For example, it becomes obvious which components could correctly reset the clock, if they were affected by light signalling pathways. Numerical simulations using such models have enormous potential relevance to molecular experiments but are rarely used in the biological community, due to their apparent inaccessibility. We are currently modelling the flowering control circuit in a package that is accessible to experimental biologists, based on the most widely-used spreadsheet programme. Funded by BBSRC and DTI.

### **C5/P3.20—Interaction of light and plastid signals mediating the early stages of chloroplast development**

C. McCormac and M.J. Terry, School of Biological Sciences, University of Southampton

In plants, the components of the chloroplast organelles (plastids) are encoded by genes distributed between chloroplast and nuclear genomes, the latter representing ancient translocation events from the plastid's autonomous genome. *Lhcb* and *HEMA1* genes of *Arabidopsis* typify such nuclear-encoded, plastid-targeted products. The *Lhcb* gene family encodes chlorophyll-binding proteins of photosystem II and *HEMA1* expression controls precursor supply for chlorophyll biosynthesis. Each is strongly upregulated via phytochrome- and cryptochro-

me-mediated light-signalling cascades but also requires a retrograde plastid signal(s), that emanates from the chloroplasts to influence nuclear transcription. We measured expression patterns during chloroplast development in de-etiolating seedlings of wild-type *Arabidopsis* and within several mutants of light-signalling components. We also measured the effects of impaired chloroplast-signalling within wild-type and genomes uncoupled (*gun*) seedlings treated with the herbicide Norflurazon or pre-irradiated with far-red light. The two transcript species showed distinct response profiles to light and plastid signalling, indicating that crosstalk between the pathways (probably at the level of the individual promoters) determines final expression. The relative dominance of each pathway's input appears compatible with the roles of the respective gene-products. We suggest that a critical product-role favours global (cell), over local (plastid) control of expression and, so, provides a paradigm for the selective pressures that have favoured the translocation of certain chloroplast genes. Thus, nuclear genes enable photoreceptor transduction pathways to drive co-ordinated cell development while crosstalk from retrograde plastid signalling attunes the response to end-point demand.

### C5/P3.21–Circadian signalling in stomatal guard cells

N. Dodd, J. Love and A.A.R. Webb, Department of Plant Sciences, University of Cambridge.

Plant circadian systems unite input (entrainment) signals that synchronise the endogenous clock with environmental cues, feedback loops that drive the core molecular oscillator, and output signals that elicit cellular and physiological responses. We have combined calcium-based imaging with physiological and molecular approaches to investigate whether circadian oscillations in cytosolic free calcium ( $[Ca^{2+}]_{cyt}$ ) function as a signal output from the clock. We will describe data suggesting that circadian  $[Ca^{2+}]_{cyt}$  oscillations can encode information relating to the light environment before and after circadian entrainment. Pharmacological and molecular tools are being used to dissect the pathway that generates circadian  $[Ca^{2+}]_{cyt}$  oscillations. Guard cells represent an attractive model system to investigate the role of  $[Ca^{2+}]_{cyt}$  as an output from the circadian oscillator since guard cell  $[Ca^{2+}]_{cyt}$  is an established regulator of stomatal aperture in response to environmental signals. We are using the guard cell system to investigate the importance of the circadian clock for the optimization of stress signalling and water use efficiency.

### C5/P3.22–ABA signalling in guard cells

L. Hunt and J.E. Gray, MBB Department, University of Sheffield; L.N. Mills, M.R. McAinsh, and A.M. Hetherington, Biological Sciences, University of Lancaster; C. Pical, Lund University

ABA is a drought-induced hormone that results in closure of pores on surfaces of leaves. The aperture of these pores is controlled by pairs of guard cells. Closure has been shown to involve the intracellular release of calcium, via a number of second messenger molecules. One of these, IP<sub>3</sub>, is produced by the hydrolysis of phospholipids by phospholipase C (PLC). Our recent results show that tobacco and *Arabidopsis* guard cells contain PLC protein and that guard cells with genetically reduced levels of PLC show altered aperture responses to ABA. The *Arabidopsis* genome contains 7 PLC isoforms, and our promoter GUS fusions show that at least one is present in guard cells. We have also expressed *Arabidopsis* PLC isoforms in *E.coli* as fusion proteins and determined their differing calcium requirements. Another calcium releasing second messenger, cADPR, and the enzyme involved in its synthesis is well characterised in mammals. An inhibitor of the enzyme responsible, ribosyl cyclase has been shown to reduce ABA-induced aperture closure. However, there are no clear ribosyl cyclase homologues in the *Arabidopsis* genome. We shall present our progress in purifying this activity from *Arabidopsis* extracts. The successful identification of a plant ribosyl cyclase gene would provide a new mechanism to study cross-talk between calcium-release pathways in ABA signal transduction. We are also trying to identify further *Arabidopsis* guard cell proteins that are induced or modified by ABA treatment using DIGE, and sequencing those proteins that change in abundance or PI using the GARNET proteomics facility.

### C5/P3.23–Expression of Sphingosine Kinase Genes in *Arabidopsis thaliana*

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Sphingosine-1-phosphate, has recently been shown to be involved in response to drought in *Commelina communis*, and is involved in guard-cell responses to ABA. The focus of our work is to investigate the enzyme responsible for sphingosine-1-phosphate production, sphingosine kinase, in *Arabidopsis thaliana*. Four putative sphingosine kinase genes have been identified in the *Arabidopsis* genome. Results will be presented on the expression patterns of these genes in *Arabidopsis*.

### **C5/P3.24—Oxidative stress signalling in *Arabidopsis***

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Oxidative stress, arising from an imbalance in the generation and removal of reactive oxygen species such as hydrogen peroxide ( $H_2O_2$ ), is common to all eukaryotes, including plants. Plants generate  $H_2O_2$  in response to various abiotic and biotic stresses such as extreme temperatures, drought, air pollutants, hormones and pathogen challenge. Similarities in cellular responses and signalling pathways highlight  $H_2O_2$  as a likely candidate for a key signalling molecule orchestrating defence responses. We have established that *Arabidopsis* cells generate  $H_2O_2$  in response to bacterial elicitors and avirulent bacteria, and that  $H_2O_2$  activates specific MAP kinase signalling pathways, defence genes and programmed cell death. Using mRNA differential display we have identified genes encoding signalling proteins up-regulated by  $H_2O_2$  treatment. In order to ascertain the global responses to  $H_2O_2$  at the transcriptome level, we performed a DNA microarray analysis of  $H_2O_2$ -modulated gene expression in *Arabidopsis* cells. We identified a number of genes both up- and down-regulated by  $H_2O_2$ , that includes genes encoding proteins involved in signalling, defence and stress responses. Using RNA blots we also show that some of these genes are induced in response to UV, elicitor and drought stress, both via, and independent of,  $H_2O_2$ . Our data place  $H_2O_2$  as an important mediator of cellular responses to various plant stresses.

### **C5/P3.25—Functional Genomics of Ozone Stress in *Arabidopsis***

E. Short, M. McAinsh, A. Shirras and T. Huckerby, Lancaster University

The gas ozone functions as a protector against ultra-violet radiation in the stratosphere. However, in the troposphere it is toxic to plants and causes significant reductions to crop yields. Ozone is a reactive oxygen species (ROS) and can cause oxidative damage directly by entering stomata and interacting with cell wall and membrane components. Ozone can also form other ROS such as hydrogen peroxide and hydroxyl radicals that can cross the plasma membrane and cause further damage, leading to reduced transpiration, accelerated senescence and decreased photosynthesis. Plants react to oxidative stress by increasing their antioxidant defences in an attempt to neutralise harmful ROS. The individual roles of these antioxidants are well understood, however their regulation and interaction in planta have yet to be fully elucidated. In this work a functional genomics

approach has been used to identify novel genes in *Arabidopsis thaliana* that are regulated by ozone. A DNA microarray has been utilised to determine gene regulation at the transcriptional level and NMR spectroscopy has been used to investigate the effect of ozone on the metabolite profile.

### **C5/P3.26—A pharmacological and mutagenic approach to dissecting the response of *Arabidopsis thaliana* to ozone**

K.A. North, N.H. Evans, A.M. Hetherington, M.R. McAinsh, Biological Sciences, Lancaster University; M. R. Knight, Department of Plant Sciences, University of Oxford

Stratospheric ozone protects against ultra violet radiation, however in the troposphere it is toxic to plants and animals, causing reductions in crop yield and forest damage. Ozone, a reactive oxygen species (ROS), can cause oxidative damage by entering stomata and reacting with cellular components. It can also form other ROS such as hydrogen peroxide and hydroxide radicals, which are able to traverse the plasma membrane causing cellular damage. Generation of these ROS in response to ozone is implicated in cell death and visible injury in many species of plant.

Plants must adapt to their environment as they are unable to move away from stress. They therefore increase their antioxidant responses following oxidative stress, this enables them to remove harmful ROS. The signal transduction pathways leading to increased defence responses is not well understood, however an early response to ozone is an increase in cytosolic free calcium  $[Ca^{2+}]_{cyt}$ . *Arabidopsis thaliana* transformed with the photoprotein apoaequorin have been utilised to measure changes in  $[Ca^{2+}]_{cyt}$  in response to ozone and following pretreatment with a range of pharmacological agents. This was to help establish the pathways involved in the propagation of the signal from ozone stimulus to response. Plants with disrupted calcium homeostasis mechanisms, also transformed with aequorin have also been used to investigate the pathways and calcium stores involved in the generation of the signal.

### **C5/P3.27—Phosphoinositide signalling and environmental stresses**

Lee, J., Hunt, L and Gray, J.E. Molecular Biology and Biotechnology, University of Sheffield

The phosphoinositide signalling pathway is a ubiquitous eukaryotic pathway involved in converting a variety of extracellular stimuli into intracellular responses. One of the enzymes involved in this pathway is phospholipase

C (PLC). The aim of the work presented here is to investigate the phosphoinositide-specific PLC's (PI-PLC). These hydrolyse phosphoinositol 4,5-bisphosphate (PIP<sub>2</sub>) into two second messengers, inositol 1,4,5-trisphosphate (InsP<sub>3</sub>) and diacylglycerol (DAG). Within the *Arabidopsis thaliana* genome we have identified nine genes for isoforms of PI-PLC. All nine isoforms have been shown to be expressed within *Arabidopsis* plants using RT-PCR, with differing expression patterns. Evidence has shown a role for PI signalling in plant responses to a variety of environmental stimuli, which include salt stress, dehydration and ABA signalling pathways. Other suggested stimuli include pathogen attack and exposure to cold. The objective of these experiments is an investigation into the induction of PI-PLC gene expression in response to various environmental stimuli, to determine which isoforms are induced in response to each stimulus.

#### **C5/P3.28—The interaction effects of Ca<sup>2+</sup>, ABA, IAA and pH on stomatal movements**

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Recent results demonstrated that pH, Ca<sup>2+</sup> and ABA may have mutually interactive effect on stomatal closure. In contrast to ABA, less is known about the signaling events involved in stomatal opening in response to the plant hormone IAA. Therefore, the aim of presented paper was to investigate the response of stomata to of pH (4–8), Ca<sup>2+</sup> (0.01–1 mM) and ABA and IAA (10 nM and 10 μM) on stomatal aperture in guard cells of *Commelina communis* L.

Stomatal aperture measurements were done in abaxial epidermal strips. Strips were incubated in a buffered solutions (pH 4–8) containing ABA (10<sup>-6</sup> or 10<sup>-8</sup> M) and exposed for 3hr to illumination (200 μmol photons m<sup>-2</sup>s<sup>-1</sup>) in CO<sub>2</sub> free air. Under the same conditions and in the same incubation medium (pH 5 or pH 7) stomatal closure was induced by different Ca<sup>2+</sup> (0.01–1mM) and ABA concentrations (10 nM and 10 μM), although stomatal opening with the same Ca<sup>2+</sup> concentrations and 10 nM and 10 μM IAA. Stomatal aperture was measured using a microscope image analyzing system.

Obtained results showed that the effect of pH, Ca<sup>2+</sup>, ABA and IAA on stomata depends on their concentrations and mutual interactions. Ca<sup>2+</sup>-dependent increase in the rate of both closure and opening followed a biphasic response, with maximal values depending on pH. ABA and IAA altered this response, with an increased rate of stomatal reactions being observed at higher hormones and lower Ca<sup>2+</sup> concentration or vice versa.

#### **C5/P3.29—The interaction effects of ABA and pH on maize leaf growth**

Z. Jovanovic, L. Prokic and R. Stikic (Belgrade, Yugoslavia)

Abstract not supplied

#### **C5/P3.30—The Control of Root Development in *Arabidopsis* by light**

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The light environment controls all aspects of plant development. A complex network of pathways involving many gene products, both identified and unidentified, arises from at least 3 families of photoreceptors. Complexity and redundancy allow plants to detect light intensity, quality and direction, and to direct growth accordingly, and this is particularly striking during the de-etiolation switch and shade avoidance response. Extensive investigation of these pathways has focused on the cotyledons and hypocotyl, and root phenotypes have often been overlooked or dismissed. Recent work has strengthened the association of light with auxin synthesis, and auxin has been implicated in many aspects of root development, including primary root elongation, lateral rooting, and root hair production. Here we present data showing distinct root phenotypes of the phytochrome null mutants, and suggest roles for the light environment in determining root phenotype.

#### **C5/P3.31—Signaling programmed cell death-ceramide biosynthesis holds life in the balance**

J.E. Markham, S.D. Spassieva, T. Gechev, M. Ferwerda and J. Hille, Department of Molecular Biology of Plants, University of Groningen

Programmed cell death is the remarkable event that occurs when a cell commits suicide. Several events may trigger plant cells to commit suicide, developmental cues such as xylem formation and leaf senescence, environmental stimuli for instance hypoxia or event light in certain species, and signals during plant–pathogen interactions. During one particular plant pathogen interaction, the fungus *Alternaria alternata* f.sp. *lycopersici*, a necrotrophic fungus unable to grow on living tissues and kills its host plant, *Lycopersicon esculentum*, by secreting a toxin, AAL-toxin, that induces programmed cell death in the host plant. Labeling experiments show that AAL-toxin causes inhibition of ceramide biosynthesis and an increase in ceramide precursors known as long chain bases. Evidence suggests that programmed

cell death is triggered, in this case, by an upset in the balance between ceramide and long chain bases as either preventing the accumulation of long chain bases, or supplying external ceramide, is able to prevent programmed cell death. Previous work has implicated ethylene and salicylic acid in AAL toxin-induced programmed cell death. We have isolated an AAL-toxin sensitive *Arabidopsis thaliana* and are using this plant line to investigate the signaling pathways involved in AAL toxin-induced programmed cell death.

### **P3/C5.32–Phospholipase C in plant signalling**

L. Hunt and J.E. Gray (Sheffield); L.N. Mills, M.R. McAinsh and A.M. Hetherington (Lancaster)

Abstract not supplied

### **P3/C5.33–Interactions between plant hormone signalling and gibberellin biosynthesis**

N.R. Clark, J.P. Coles, O. Ruiz, P. Hedden and A.L. Phillips, Rothamsted Research

The gibberellin family of plant hormones is involved in a number of different aspects of plant growth and development. There is evidence that both GA biosynthesis and the GA signal transduction pathway are modulated by developmental events, environmental factors and by other plant hormones. A key regulatory step in GA biosynthesis is catalysed by GA 20-oxidase (GA20ox), a 2-oxoglutarate-dependent dioxygenase that in *Arabidopsis* is encoded by a gene family of five members. Expression of *GA20ox* genes is down-regulated by bioactive GAs in a feedback mechanism that maintains GA homeostasis. We are using reporter constructs, consisting of the *Arabidopsis GA20ox1* promoter and coding region, up to the start of exon 3, fused to GUS and LUC. Analysis of transgenic *GA20ox1::GUS* lines shows that the gene is expressed in the shoot apex, young leaves, petioles, cauline leaves and pollen. Expression of the reporter genes is feedback regulated by GA and we have screened M2 populations of EMS-mutagenised seeds of the *GA20ox1::LUC* lines to identify mutants with altered feedback regulation. We have also studied the effect of exogenous application of hormones on the expression of *GA20ox1* and we are introducing the transgenes into a range of hormonal and developmental mutant backgrounds to study the interaction between these pathways and GA biosynthesis.

### **C5/P3.34–New lipid-derived signals in plants and their role in modulating gene expression**

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We have examined the role of two classes of lipids in signalling and modulating gene expression. Firstly, we have identified a new octadecanoid fatty acid metabolite signal that is distinct from the previously described methyl-jasmonate pathway. This compound, *cis*-jasmonone, not only acts as an insect semiochemical but also induces a suite of plant genes. Transcriptome analysis indicates a relatively small number (~30) of genes upregulated by *cis*-jasmonone. These include a number of genes involved in cell wall biosynthesis, as well as other, as yet undefined, functions. Interestingly, even though *cis*-jasmonone is hypothesised to be a metabolite of jasmonic acid, there is no overlap in the suite of genes upregulated by either *cis*-jasmonone or methyl-jasmonate. We have constructed promoter-luciferase fusions to further examine the signalling pathway of *cis*-jasmonone. Secondly, we have examined the role of sphingolipid long chain base (LCB) heterogeneity in plants; unlike animals or yeast, plants have considerable variation in their LCB composition. In animals, the predominant LCB sphingosine is the precursor of sphingosine-1-phosphate, the well-documented dynamic signalling molecule. The role of sphingosine in plants and fungi is less clear, though recent work has implicated S-1-P in guard cell closure. We have identified the single gene responsible for the synthesis of sphingosine in Fission yeast and deleted it by gene targeting. Surprisingly, inactivation of this gene (the dihydroceramide desaturase) has no effect of cell viability. This may imply that the role of sphingosine and its metabolites (i.e. S-1-P) differs in low eukaryotes from that observed in animals.

### **C5/P3.35–The tomato 14-3-3 protein gene family—specificity or redundancy?**

G.L. de Bruxelles, A.P. Brown and M.R. Roberts, Biological Sciences, Lancaster University

14-3-3 proteins regulate a wide range of target proteins via direct protein–protein interactions. One of their possible functions is to act as adapter molecules mediating interactions between signalling proteins. A common idea put forward in the literature is that heterodimers containing different combinations of 14-3-3 isoforms might therefore regulate signalling cross-talk. In apparent opposition to this idea, however, is the observation that the target-binding domain in 14-3-3 proteins is highly conserved, suggesting similar biochemical properties for

all 14-3-3s. Despite this, higher eukaryotes possess multiple 14-3-3 genes, and these genes exhibit diverse patterns of gene expression within tissues and in response to stress. This tends to suggest specific functions for particular genes. Some biochemical data also suggest 14-3-3 isoform-specific protein–protein interactions, whereas other studies conclude that apparent isoform-specificity is simply the result of differences in expression patterns rather than in the biochemical properties of 14-3-3 isoforms. Thus, the understanding of whether and how 14-3-3s exhibit isoform-specific functions is a

key question. Here we present evidence that despite the wide range of processes in which they are involved, inhibition of expression of individual 14-3-3 isoforms results only in minor phenotypic changes in tomato plants. However, we also demonstrate that at the level of biochemical function, different isoforms possess distinct target-binding properties. We argue that apparent redundancy at the genetic level is partly a consequence of isoform-specificity being imposed by quantitative rather than qualitative differences in affinities for target proteins.