Meeting report

Signaling in plants
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A report on the 52nd Harden Conference on 'Signaling in Plants', Wye College, Kent, UK, 18-22 September 2000.

This meeting was organized jointly between The Biochemical Society and *The New Phytologist* and brought together an international field of plant scientists to discuss some of the latest advances in the study of signal transduction in plants. In this short report we have highlighted only some of the areas reported. It became increasingly evident during the meeting that 'cross-talk' and integration between plant signaling pathways will become an even more important theme in the future.

Calcium signaling

There is now good evidence that plant cells not only contain key components of the calcium-mediated signaling pathways found in animal cells but also use them. One powerful experimental system to study calcium signaling mechanisms has been the stomatal guard cell complex, which can be induced to open by various effectors such as high light levels, low CO_2 concentrations, auxins, cytokinins and fusicoccin, and can be induced to close by abscisic acid (ABA), high CO_2 and inositol trisphosphate ($InsP_3$). A common feature of these activities is the involvement of calcium ions. For example, the experimental addition of ABA to guard cells leads to a heterogeneity in calcium levels across the cell - a 'calcium signature' - that can be visualized by confocal microscopical imaging techniques (Alistair Hetherington, Lancaster University, UK). Hetherington argued that it is the uniqueness of each signature that represents the cellular expression of calcium signaling specificity. A number of interesting questions were raised at the conference. What range of molecules act as effectors of calcium signatures? How are distinctive calcium signatures generated? And how do cells interpret or decode different calcium signatures?

Charles Brearley (University of Cambridge, UK) showed evidence for the role of inositol hexakisphosphate ($InsP_6$) as an intermediate component in the ABA-induced calcium transients and guard cell closure of the potato (*Solanum tuberosum*). $InsP_6$ is very effective (more so than $InsP_3$) at inhibiting potassium influx and guard-cell opening, although this depends on the $InsP_6$ conformer (*myo*-inositol is effective, *scyllo*-inositol is not). Hetherington showed that sphingosine-1-phosphate, which is known to be involved in several aspects of animal cell signaling (including for example cell motility and proliferation, and cytoskeletal function) can induce calcium oscillations in guard cells and induce closure; the inactive analog dihydrosphingosine-1-phosphate has no such effect.

Where does the calcium transient originate? Does $InsP_3$ or $InsP_6$ mobilize internal calcium stores, or induce calcium influx across the plasma membrane? It seems possible that the nature of a calcium signature could be determined by the dynamics of calcium influx and release from various subcellular compartments. Dale Sanders (University of York, UK) described the use of patch clamping to demonstrate how $InsP_3$ and cyclic ADP-ribose can induce calcium release from single isolated vacuoles. In contrast to the success in animal and yeast systems, however, no unequivocal candidate for a plant calcium channel has been identified or cloned. Once this is achieved, the molecular basis of the generation of unique calcium signatures should become clearer.

Marc Knight (Oxford University, UK) described some of his group's work on how calcium signaling is involved in sensing temperature changes during chilling and freezing. Of interest was the finding that the magnitude of cytosolic calcium increases in response to temperature lowering depends on the rate of cooling and not on the absolute temperature. Again, one theme was the question of how calcium signatures can encode specificity to downstream signaling events.

Plant hormones

Although ethylene signaling is now the best understood of the so-called classical hormonal systems in plants, auxin is hot on its heels. Most advances in recent years have been a consequence of the success of the genetic approach in identifying mutants that are either resistant to auxin, overproduce it, or fail to transport it around the plant correctly. An increasing number of auxin-responsive genes have been identified, and candidate auxin receptors have been purified and the genes cloned. Ottoline Leyser (University of York, UK) described progress in the construction of a model to account for the diversity of auxin action, and focused in particular on the insight generated by auxin-resistant mutants of *Arabidopsis*, including *axr3*, which is a member of the Aux/IAA family of auxin-responsive genes. AXR3 is normally degraded rapidly, but gain-of-function *axr3* mutants are mutant in an amino-terminal region required for degradation, leading to abnormally stable AXR3 protein and an 'auxin over-responsive' phenotype. Other domains on the AXR3 protein are likely to be involved in the formation of homodimers and heterodimers, in the latter case with ARF transcription factors, to contribute to the observed diversity of auxin action. Paul Millner (Leeds University, UK) discussed a model for the role of heterotrimeric G proteins in auxin signaling, in which the action of the auxin-binding protein (ABP), a likely auxin receptor, is mediated by an ABP-specific G-protein-coupled receptor.

Of the more recently identified signaling systems in plants, jasmonate (JA) and brassinosteroids received some attention. John Walker (University of Missouri, Columbia, USA) illustrated the value of the use of activation tagging screens in unraveling the brassinosteroid signaling pathway in *Arabidopsis*. *BRI1* encodes a predicted receptor kinase, mutations in which lead to a brassinosteroid-insensitive phenotype. Activation tagging led to the identification of a *bri1* suppressor, *brs1*, and the BRS1 gene was found to encode a serine carboxypeptidase. Such proteins appear to be involved in preprotein processing, and it is possible that rescue of *bri1* requires high levels of BRS1 to promote either correct receptor processing or enhanced ligand delivery to the receptor.

JA can, like auxin, influence root development, and in particular can inhibit root elongation. John Turner (University of East Anglia, Norwich, UK) has used a genetic approach to identify new components in the JA signaling pathway of *Arabidopsis*. For example, the *coi1* mutant is resistant to growth-inhibitory concentrations of the JA coronatine, and *coi1* encodes an F-box protein with leucine-rich repeats. Yeast two-hybrid protein interaction screens have identified coi1-interacting proteins; no data on these were presented. A second type of screen led to the identification of mutants putatively with defects in the JA gene activation system. The vegetative storage protein (VSP) gene promoter is JA-inducible, and the *cev1* mutant shows constitutive VSP-luc expression in the absence of JA. It is also defective in various aspects of root growth and, significantly, has enhanced resistance to the aphid *Myzus persicae*, consistent with a role for JA in plant defense against insects.

Active oxygen species

There is great interest in how active oxygen species (AOS) are perceived by signaling pathways and how they act as messengers. In response to pathogen attack, plants undergo an oxidative burst which is believed to be similar to that observed in animal immune responses involving phagocytes. There is a great deal of uncertainty as to the exact mechanism of AOS generation in plants. Plants do contain homologs of the mammalian NADPH oxidase gp91[phox], which is a candidate for AOS production. But Paul Bolwell (Royal Holloway and Bedford New College, Egham, UK) provided evidence for an alternative mechanism of AOS production utilizing extracellular peroxidases, alkalinization and release of substrate, and he suggested that the relative contribution of different AOS generating systems may vary from species to species. The poster by Vanacker and co-workers (Institute of Arable Crops Research, Rothamstead, Harpenden, UK) reported that barley lines resistant to infection by powdery mildew accumulated both glutathione and catalase after hydrogen peroxide production in response to infection. One possible mechanism for this was presented by Alice Harmon (University of Florida, Gainesville, USA) who overviewed the field of calcium-dependent protein kinases (CDPKs). These are restricted to plants and protozoa, and one soybean isoform phosphorylates and changes the activity of serine-acetyltransferase, an enzyme of cysteine, and hence glutathione, biosynthesis. Some CDPKs are activated by pathogen attack and Jonathan Jones and co-workers (John Innes Centre, Norwich, UK) presented data showing that plants with a specific CDPK gene silenced showed a retarded hypersensitive response.

The mechanism(s) of systemic acquired resistance (SAR) to pathogens is now also falling under the scrutiny of genetic screens. Chris Lamb (John Innes Centre, Norwich, UK) described the *dir1* mutant of *Arabidopsis* that is defective in SAR. Over-expression of the DIR1 protein, which encodes a small lipid-transfer-protein-like polypeptide, leads to a dramatic enhancement of SAR. A model was discussed in which DIR1 acts as a chaperone or transporter of the mobile signaling component of SAR, rather than forming the signal itself; the signal, it was speculated, could be a lipid derivative possibly produced during the oxidative burst.

Russel Jones (University of California, Berkeley, USA) discussed the interplay of hormones in the control of apoptosis of aleurone cells in barley endosperm. Using protoplasts as a model system, his group found that gibberellic acid (GA) induces cell death whereas ABA maintains cell viability (for up to 6 months). GA-treated cells accumulate hydrogen peroxide, which will kill such cells either as protoplasts or intact

cells. In contrast, ABA-treated cells are not susceptible to cell death by hydrogen peroxide, and this correlated with increased levels of AOS-detoxifying enzymes such as catalase, superoxide dismutase and ascorbate peroxidase (all of which are down regulated in GA-treated cells). In this system hydrogen peroxide (possibly produced by β-oxidation of fatty acids) is likely to cause cell death directly, as opposed to being the messenger of the apoptotic signal. Jones suggested that nitric oxide (NO, a recent addition to the list of messengers in plant cell signaling) may have a role in the control of apoptosis, as NO donors can prevent cell death.

Novel systems and components

Sheng Luan (University of California, Berkeley, USA) described the molecular characterization of plant protein-tyrosine phosphatases, a previously untapped area of research in plants. One tyrosine-specific protein phosphatase (PTP) was shown to affect the phosphorylation status of a mitogen-activated protein (MAP) kinase. A second phosphatase that was described, a dual-specificity phosphatase (dsPTP), appears to be involved in the control of pollen development. This dsPTP shares most similarity over its catalytic domain to PTEN, an animal tumor suppressor, which hydrolyzes inositol phospholipids. In plants, however, the *in vivo* substrates of PTPs and dsPTPs remain elusive at present. This work also demonstrated the use of RNA-mediated interference (RNAi) to repress the expression of specific genes. Higher plants are not yet amenable to routine gene knockouts using homologous recombination, but M. Gonneau and co-workers (Institute National de la Recherche Agronomique, Versaille, France) presented data on the use of such methods in the moss *Physcomitrella patens*.

Carol MacKintosh (University of Dundee, UK) presented elegant work on the identification of phosphoproteins that bind to 14-3-3 proteins, which are highly conserved phosphopeptide-binding proteins. Using affinity purification techniques coupled with mass spectrometry, numerous key metabolic enzymes and signaling proteins were identified as 14-3-3 targets. Of great interest was the finding that 14-3-3 binding was dependent upon the presence of sugar; in its absence, 14-3-3 binding was lost and the target proteins were subject to specific proteolytic cleavage. These findings have important implications both for the integration of plant cell metabolism and for the study of disease states in animals, where nutrient supply can have effects on hormonal responses.

Perspectives

One clear theme to emerge from this meeting was the need to integrate both genetic and biochemical approaches to study plant signal transduction. Although the use of mutants has been a powerful technique for identifying genes encoding components of signal transduction pathways, the way forward will clearly be to identify interacting proteins as, by analogy with animal systems, it is likely that individual components will be organized into supramolecular complexes. The technical advances being made in proteomics and genomics, coupled with mutant screens and more 'classical' biochemical approaches, are going to have a radical impact upon our understanding of how plants perceive and respond to diverse signals.