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# Cell signalling and gene regulation
# Signalling mechanisms in plants: examples from the present and the future
## Editorial overview
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The application of molecular genetics and the availability of whole-genome sequences have revolutionized our understanding of signalling mechanisms in plants. Subsequent biochemical analysis of the proteins that have been identified has revealed the identity of the receptors controlling responses to most growth regulators and of several of the photoreceptors that confer responses to light. In several cases, unexpected biochemical functions have been uncovered. For example, the ubiquitin ligase TIR1 is required for the degradation of transcriptional repressors of auxin responses and acts directly as the auxin receptor [1,2]. The blue-light receptor FLAVIN-BINDING, KELCH REPEAT, F-BOX 1 (FKF1) has also been found to be a ubiquitin ligase [3]. These examples illustrate the novelty of the signalling mechanisms uncovered in plants compared to those already described in animal systems. Connections between the functions of growth regulators and the activities of transcription factors that have important roles in growth and development have recently been established, thereby relating hormonal signalling mechanisms to important transcriptional changes. Furthermore, the widespread importance of novel transcriptional regulatory mechanisms, including small RNAs and epigenetic regulation by histone modifications, has become apparent. By contrast, the molecular mechanisms underlying other important plant responses, such as how plants detect and respond to ambient temperature, are much less well understood. In this issue of *Current Opinion in Plant Biology*, we focus on examples of signalling and gene regulation where striking progress has been made in recent years, and also highlight other cases where the mechanisms are not yet well understood but that represent areas to watch in the years to come.

For plants, light is both a source of energy and of information that allows the modification of their growth and development to suit the ambient environment. For example, competition for light determines the success of individual plants in dense vegetation and shading by neighboring plants triggers a complex developmental response called shade-avoidance. Although red (R):far red (FR) ratios and phytochromes have been identified as the main players in shade-avoidance responses, blue light and hormone regulators have also emerged as important factors determining elongation of the stem. Vandenbussche *et al*. review recent developments in this field, placing particular emphasis on hormonal control of stem growth. Low R:FR ratios are redundantly detected by light-stable phytochromes that migrate to the nucleus upon photoconversion. There, they interact with different PROTEIN INTERACTING FACTOR (PIF)/PIF-like (PIL) basic helix–loop–helix (bHLH) transcription factors and so regulate the expression of genes such as those encoding the homeobox HD-ZIP proteins ATHB-2 and

ATHB-4. Blue-light receptors cryptochromes and photo-tropins are also involved in shade avoidance. Interestingly, mutual interactions have been detected between blue-light receptors and phytochromes, which act together to control responses to shade. The enhanced hypocotyl elongation caused by reduced R:FR is day-length-dependent. PIL1 has been shown to interact with the clock gene oscillator TOC1 and to exhibit circadian regulation, acting as a possible molecular link between shade and clock-regulation. Vandenbussche *et al*. also discuss roles for auxins, ethylene and brassinosteroids in shade avoidance, pointing to hormonal crosstalk as an important factor that regulates shade avoidance in plants.

The review by Hoecker focuses on the importance of ubiquitin-mediated proteolysis in light signalling. The CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) ubiquitin ligase is required to repress light signalling and photomorphogenesis in the dark. COP1 interacts with transcription factors, such as LONG HYPOCOTYL 5 (HY5), LONG AFTER FAR-RED LIGHT1 (LAF1) and LONG HYPOCOTYL IN FAR-RED1 (HFR1), that are involved in photoreceptor signalling and promotes their degradation in the dark. Furthermore, COP1 has additional roles in the degradation of the photoreceptor PHY-TOCHROME A (PHYA) in response to light and, surprisingly, in the stabilization of the bHLH transcription factor PIF3 in the dark. The biochemical mechanism by which COP1 acts is not clear but appears to involve a large protein complex that includes other proteins that were identified genetically as repressors of photomorphogenesis, such as DEETIOLARED1 (DET1) and SUPPRESOR OF PHYTOCHROME A-105 1 (SPA1). The recent demonstration that COP1 acts in a related complex in human cells suggests interesting parallels between photoreceptor signalling in plants and ubiquitination in vertebrates.

Intensive studies carried out over many years have led to the identification of the photoreceptors and signalling molecules that mediate responses to blue, red and far-red light. By contrast, the mechanisms that control responses to UV-B light are still largely unknown despite UV-B having important effects on plants, both as an environmental stress and as a developmental signal. The review by Ulm and Nagy describes how the application of whole-genome transcript profiling and genetics to UV-B signalling has enabled important advances in this field. The *Arabidopsis* genes that are induced in response to short periods of exposure to UV-B were identified, and genes that encoded transcription factors comprised 20% of these. One of these transcription factors, HY5, also plays a major role in cryptochrome and photoreceptor signalling, and impairment of this protein resulted in reduced expression of a sub-set of UV-B-responsive genes. Performing forward genetics to identify more mutations that prevent the upregulation of these genes

and using reverse genetics to inactivate other genes that are induced by UV-B represent promising approaches to determining the mechanisms of UV-B light signalling.

Plants are also sensitive to changes in ambient temperature, but the mechanisms involved in the perception of and response to this signal have not been identified. Samach and Wigge describe some of the molecular mechanisms that perceive changes in ambient temperature in bacteria, and point out that although no temperature-sensing molecules have been described in plants, pathways that are affected by ambient temperature control processes such as flowering and circadian-clock regulation. These observations form a strong basis for further studies of the mechanisms that underlie responses to this intriguing environmental signal.

The arrangement of leaves, flowers and floral organs in regular patterns has long caused interest because of its peculiarity in following mathematical rules characterized by the Fibonacci numbers. Mutants that are altered in auxin biosynthesis, perception or transport exhibit defects in organ position, indicating that auxin has an important role as a morphogen in phyllotaxis. Current models of phyllotaxis are discussed by Reinhardt. According to these models, the position of the new primordium is mainly determined by pre-existing primordia, which, by actively accumulating auxin, cause auxin depletion in their vicinity. The future organ is initiated at points that are out of the influence of this auxin-depleting activity, with default accumulation of auxin at these points inducing PIN-FORMED1 (PIN1) expression and early founder cell identity. The influx carrier AUX1 and the efflux carrier PIN1 are involved in active auxin accumulation, with the protein kinase PINOID (PID) being an important determinant of asymmetrical PIN1 localization. The auxin response factor (ARF) gene *MONOPTEROS* (*MP*) is involved in downstream signal transduction of the auxin signal and in subsequent activation of the regulatory pathways leading to organ formation.

In addition to being involved in organ development, polar auxin transport provides essential directional and positional information for developmental processes such as vascular differentiation, apical dominance, and tropic growth. On a cellular level, directional auxin transport is primarily controlled by the efflux carrier complex comprised of the PIN family of proteins. Influx carriers are less important in directional transport because indole-3-acetic acid (IAA) can enter the cell by diffusion through the plasma membrane. Blakeslee, Peer and Murphy review the current state of knowledge concerning polar auxin transport, the contributing roles of the auxin influx (AUX1/LAX) and efflux carriers (PIN1–PIN7), and the molecular mechanisms involved in regulating these membrane transporters. Plant orthologues of mammalian multidrug-resistance/P-glycoproteins (MDR/PGPs) have also

been shown to play an essential role in auxin transport. PGPs function in the ATP-dependent movement of hydrophobic substrates, and co-purify with glycosylpho-sphatidylinositol (GPI)-anchored proteins and PINs. Hence, it is likely that PGPs mediate the ATP-dependent transport of IAA in conjunction with PIN proteins, thus conferring further directionality and substrate specificity to the efflux complex.

A further important issue is how cell fate is determined within the primordium. Failure to establish proper leaf identity is expected to result in seedlings that have defective primary leaves. Such mutants have indeed been identified, the best characterized of them carrying mutations in a group of genes called *LEAFY COTYLEDON* (*LEC*). Loss-of-function mutations in these genes cause embryonic leaves or cotyledons to develop as rosette leaves, whereas *LEC* overexpressers show embryonic characters in the leaves. Lumba and McCourt outline recent developments in our understanding of LEC function, demonstrating that the *LEC* genes induce embryonic characters by regulating responses to abscisic acid (ABA) and gibberellic acid (GA). These hormones often act antagonistically and the ABA/GA ratio more than the concentration of each hormone most likely plays a role in the establishment of leaf identity. How changes in the ABA/GA ratio are transduced to regulate leaf identity is still unclear.

Another major determinant of the final form of a plant is the formation of secondary axes of growth from lateral meristems. Schmitz and Theres describe how this process is regulated at two levels: the initiation of the formation of lateral meristems and their outgrowth to form lateral branches. Genetic analyses of these processes suggest that the underlying mechanisms are largely conserved in diverse plant species. Two pathways appear to control the initiation of lateral meristems: one is defined by the involvement of a transcription factor of the GRAS family in *Arabidopsis*, tomato and rice, whereas a second pathway involves interactions between MYB and bHLH transcription factors, as suggested by comparative analyses in tomato and maize. Whether the meristems that are formed in response to the products of these genes actually grow out to form side branches appears to involve a new mobile signal that is likely to be a form of carotenoid. The biochemical pathway required for the synthesis of this signal was defined by genetic analysis in *Arabidopsis* and pea, and seems to be highly conserved between these species. The study of lateral meristem formation and outgrowth of side branches strongly emphasises the value of comparative studies between diverse model systems because of the accessibility of different levels of regulation in distinct species.

The development of plant organs is intimately associated with the development of vascular bundles that are required to ensure connection between all parts of the plant. The plant vascular system is composed of two types of tissues, xylem and phloem, which originate from the procambium vascular meristem. Cells in the procambium can differentiate to form either phloem or xylem. Regulatory mechanisms that are involved in the phloem versus xylem decision are summarized in Carlsbecker and Helariutta's contribution, in which an emerging pathway of control of vascular-tissue specification is discussed. Patterning of the vascular bundles in the shoot is closely associated with the adaxial/abaxial patterning of the lateral organs, with the class III *HD-ZIP* genes *REVOLUTA* (*REV*), *PHABULOSA* (*PHB*) and *PHAVOLUTA* (*PHV*) and the *KANADI* (*KAN*) genes being required in this process. Brassinosteroid (BR)-deficient mutants form increased amounts of phloem and reduced amounts of xylem, providing evidence that these hormones have a role in xylem differentiation. Genetic data support a function of the *HD-ZIPIII* genes in BR-mediated xylem proliferation. Phloem differentiation, on the other hand, requires activation by the MYB–coiled–coil transcription factor *ALTERED PHLOEM DEVELOPMENT* (*APL*), which is specifically expressed in the phloem. Xylogen, a small arabinogalactan/non-specific lipid transfer protein (nsLTP) protein that is localized in the apical side of the immature tracheary element cell walls, further contributes to finish vascular strand formation by mediating cell–cell interactions and inducing continuity of the vascular strands. Together, these findings define a well-established series of events that determines the differentiation of vascular strands, although the exact role of auxin signalling in this vascular-tissue-specification pathway remains to be determined.

Cytokinins influence many aspects of plant growth and development. Ferreira and Kieber illustrate recent developments in understanding cytokinin signalling, with particular emphasis on developmental processes recently shown to be affected by cytokinins. In *Arabidopsis*, cytokinin receptor kinases are encoded by three genes that have distinct but overlapping functions. The Histidine phosphotransfer proteins that act downstream of these receptors are encoded by five ubiquitously expressed genes. Single or double mutations in these genes do not show a phenotype, but the quintuple *ahp1 ahp2 ahp3 ahp4 ahp5* mutant is severely impaired in cytokinin response. The next step in the pathway involves *Arabidopsis* response regulators (ARRs). Type-A response regulators are rapidly induced in response to exogenous cytokinin and function as negative regulators of the response pathway. By contrast, Type-B ARRs function as transcriptional activators and bind a consensus DNA element found in the promoters of many of the cytokinin primary response genes. Perturbation of cytokinin function by triple knockout mutation of the cytokinin receptor genes leads to plants that are severely impaired in growth. There is also evidence that cytokinin might relay the

nutritional status of the plant. Nitrogen replenishment of nitrogen-starved plants leads to increases in cytokinin transport from the roots. Cytokinins block the induction of genes that are upregulated in response to phosphate starvation and repress the expression of sulphate transporter genes acting as negative regulators of sulphate uptake in sulphate-replete conditions. These findings suggest a role for cytokinin in modulating shoot growth/root growth ratio in response to different carbon/nutrient ratios. The multiple loss-of-function mutants currently available are excellent tools with which to define the function of cytokinins in the nutritional homeostasis of the plant.

The steroid hormone BR regulates many aspects of plant growth and development. The review by Li describes recent advances in understanding the BR signal transduction pathway from the transmembrane receptor to transcriptional changes in the nucleus. Perception of BR is mediated by its direct binding to a defined extracellular segment of the BRASSINOSTEROID-INSENSITIVE 1 (BRI1) protein, which triggers a phosphorylation cascade that results in the movement of the bri1-EMS-supporessor 1 (BES1) and BRASSINAZOLE-RESISTANT 1 (BZR1) transcription factors to the nucleus. These proteins both activate and repress the transcription of BR-regulated genes and, in the case of BES1, this involves heterodimerisation with bHLH transcription factors. The detailed biochemical information already available to describe this pathway brings into sight the possibility of explaining the full signal transduction chain of brassinosteroid from perception at the cell membrane to the regulation of transcription in the nucleus.

Throughout their lives, plants face attack by many different pathogens that use specific invading strategies. As a consequence, they have evolved a complex network of defence-signalling pathways whose differential activation allows them to adjust responses to individual pathogens. Defence responses involve coordinated interaction of the jasmonate (JA), ethylene (ET), salicylic acid (SA) and abscisic acid signalling networks, and recent research in Arabidopsis has uncovered several key players that regulate crosstalk between these pathways. Lorenzo and Solano summarize recent progress in deciphering the involvement of the ETHYLENE RESPONSE FACTOR 1 (ERF1) and AtMYC2/JASMONATE INSENSITIVE 1 (JIN1) transcription factors in positive and negative interactions between the JA and ET pathways. ERF1 activates gene expression in response to fungal pathogens but prevents JA-mediated induction of wound-response genes. In contrast AtMYC2, activates the expression of wound-induced genes and represses the expression of pathogen defence genes. A similar negative cross-talk regulation has been observed between JA responses and the SA-induced transcription factor WRKY70. Interplay between transcription factors therefore mediates selective defence gene activation in

response to different pathogens. Protein stability also plays an important role in this signalling network, as illustrated by the discovery that three independent JA-signalling genes, i.e. CORONATINE INSENSITIVE 1 (COI1), SGT1b/JAI-4 and AUXIN RESISTANT 1 (AXR1), encode components of the ubiquitin-proteasome pathway. The F-box protein COI1 is required for all JA-dependent responses tested to date. COI1 is present in a functional E3-type ubiquitin ligase and is a specific component of the JA pathway, whereas SGT1b/JAI-4 and AXR1 are components of other pathways. Interestingly, the closest Arabidopsis homologue of COI1 is TIR1, which was recently shown to be an auxin receptor [1,2]. Therefore, it is possible that COI corresponds to the JA-receptor, consistent with the prevalent role of COI in all studied JA responses.

Studies of the early events that follow pathogen recognition have established the importance of mitogen-activated protein kinase (MAPK) cascades in plant defence signalling. Recent advances in MAPK defence signalling cascades in tobacco, Arabidopsis, and tomato are reviewed by Pedley and Martin, who outline how these signalling cascades are conserved in all three plant species. The expression of inducible and constitutively active forms of the MAPK genes has been instrumental in demonstrating their role in defence, and has also shed light on the signal transduction pathways that mediate ethylene biosynthesis in response to stress. In addition to regulating ethylene production, defence-related MAPK cascades play significant roles in the generation of reactive oxygen species (ROS) and in gene transcription. Several MAPKs translocate to the nucleus after activation and to lead to rapid activation of several WRKY genes, thus some of the molecular mechanisms that underlie regulation by these signalling cascades are beginning to be understood. An important aspect to be resolved is how response specificity is maintained, given the large number of MAPK-related proteins in plants. The expression of constitutively active forms or the silencing of these genes will surely provide an answer to this mechanistically important question in the near future.

The reviews by Willmann and Poethig and by Schubert et al. focus on mechanisms of transcriptional regulation whose importance in plants has only recently become clear. Willmann and Poethig describe how two classes of small RNAs, microRNAs (miRNAs) and short interfering RNAs (siRNAs), have roles in transcriptional regulation. In particular, they review how siRNAs were until recently thought to be involved specifically in controlling transposon activity and viral defence, but how recent experiments have demonstrated that genes required for the biogenesis of siRNAs have important roles in controlling the transition from the juvenile to the adult phase of vegetative development. The mechanisms by which siRNAs regulate this transition is not yet clear, but

Willmann and Poethig present several models that propose how siRNAs could promote juvenile development.

Schubert *et al*. describe the roles and mechanisms of epigenetic regulation of gene expression by plant homologues of *Drosophila* Polycomb-group (Pc-G) proteins. Initially, these proteins were thought to regulate relatively few genes in plants, but redundancy between related proteins disguised their widespread importance. In animal systems, the biochemical mechanism by which Pc-G proteins regulate gene expression involves the methylation of histones. In plants, mutations in specific Pc-G homologues also cause specific changes in the methylation of histones that are bound to target genes. There also seem to be differences between the plant and animal systems, however, because the mechanisms that recognise methylated histones and that repress transcription in response to their presence seem not to be conserved. Schubert *et al*. speculate on the identity of the plant proteins that might interpret these histone marks and so mediate the repression of transcription.

The reviews in this issue describe many of the advances that have recently been made in understanding signalling and gene regulation in plants. Despite our deepening understanding of the biochemical mechanisms that underlie many of these processes, the contributors have highlighted interesting examples in which our mechanistic understanding is still at an early stage.

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