Small Molecules On the Move: Homeostasis, Crosstalk, and Molecular Action of Phytohormones

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The entire development of a plant and its interaction with the biotic and abiotic environment is essentially controlled by phytohormones. The underlying control mechanisms are not well understood, if at all. In spite of intensive research in classical plant physiology, knowledge in this field is not enough for understanding and manipulating economically relevant mechanisms of plant development, defence reactions or crop yield. The combination of classical plant physiology with powerful genetic and cell biology tools has brought substantial progress in understanding single aspects of phytohormone homeostasis, perception, signal transduction, or influence on gene expression. It was this concept that attracted the German National Science Foundation (DFG) to launch a priority programme in 1999 on the molecular analysis of phytohormone action. This issue of *Plant Biology* summarizes some of the highlights from this co-ordinated research programme and thus contains an update on selected aspects of the fast-growing field of phytohormone research, especially with regard to auxin, cytokinin, gibberellin (GA), abscissic acid (ABA), jasmonate (JA), and brassinosteriods.

For non-specialists in that field, the most intriguing feature of gibberellins is their enormous chemical complexity. Pimenta Lange and Lange (2006) review work on gibberellin (GA) homeostasis with special emphasis on pumpkin as a model system. They focus on the final part of the GA biosynthetic pathway, which is catalyzed by GA oxidases. Recently, they identified regulatory sites of GA biosynthesis during plant development, including the hypocotyl and root of seedlings, and identified two distinct GA biosynthetic pathways operating in developing seeds and seedlings. Using *Arabidopsis* as a genetic model, over-expression of genes encoding pumpkin GA oxidases helped to unravel their function in the modulation of plant development. Using this approach, 7-oxidation and 3-oxidation steps (in addition to 20-oxidation) were identified to be rate-limiting for GA biosynthesis in *Arabidopsis* (Pimenta Lange and Lange, 2006).

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Significant progress has been made in understanding the brassinosteroid signalling cascade. The terminal end of the cascade consists of transcription factors which regulate expression of numerous genes. After providing an overview of the signal transduction from the plasma membrane to the nucleus, Müssig et al. (2006) describe their approaches to identify brassinosteroid-regulated genes, and highlight the functional characterization of two genes, *OPR3* and *EXO*. The OPR3 protein was shown to be required for jasmonic acid biosynthesis. The brassinosteroid-induced expression of genes such as *OPR3* and *LOX2 (lipoxygenase 2)* indicates that brassinosteroids may affect oxylipin biosynthesis under specific conditions. *EXO (EXORDIUM)* over-expression promoted vegetative growth and resulted in stronger expression of genes involved in growth processes in *Arabidopsis*. Thus, the EXO protein likely promotes growth via the modification of gene expression patterns. Its molecular mode of action is hitherto unknown and the subject of current studies (Müssig et al., 2006).

Jasmonates are potent fatty acid-derived regulators of plant defence reactions and development. A crucial step in jasmonate biosynthesis is catalyzed by the allene oxide cyclase (AOC). In Delker et al. (2006), the central role of AOCs in jasmonate biosynthesis of *Arabidopsis thaliana* is reviewed, covering aspects of biosynthesis, regulation, metabolism, and mutant properties. The work of this group led to detailed information on the role of the four AOCs in plant development. Enzymatic properties of the recombinant proteins, expression studies, tissue-specific occurrence, and inspection of promoter-GUS lines for all four *AOCs* indicate redundant and non-redundant functions during development. The *AOC* promoter activities correspond to expression of jasmonate-responsive genes in distinct tissues and suggest a potential crosstalk between jasmonates and auxins in the regulation of root growth. During flower development, *AOC* promoter activities in distinct tissues correspond to phenotypes known for mutants of jasmonate biosynthesis and jasmonate signalling. Obviously, the four AOCs offer a spatial and temporal fine-tuning in the regulation of jasmonate homeostasis during plant development (Delker et al., 2006).

Both salicylic acid and jasmonic acid are crucial compounds for defence responses in plants (Rosahl et al., 2006). To address the role of these phytohormones for pathogen defence in potato, these authors chose transgenic approaches. Potato plants expressing the *NahG* gene encoding salicylate hydroxylase

were unable to accumulate salicylic acid in response to pathogen infection or elicitor treatment. Despite reports in the literature that salicylic acid is not required for defence against *Phytophthora infestans,* the causal agent of late blight disease, these authors could determine significantly higher pathogen biomass in *NahG* plants compared to wild-type plants. In order to specifically address the role of jasmonic acid biosynthesis for defence of potato against microbial pathogens, they generated RNA interference constructs for the genes encoding the jasmonic acid biosynthetic enzymes AOC and OPR3. Transgenic potato plants had highly reduced *AOC* or *OPR3* transcript levels after wounding and elicitor treatment, as well as highly reduced jasmonic acid levels. *P. infestans* could grow to significantly higher levels on transgenic OPR3-RNAi plants than on wild-type plants, as measured by real-time PCR; however, pathogen growth was not as high as in *NahG* plants. These data suggest that not only salicylic acid but also, albeit to a lower extent, jasmonic acid is of importance for basal defence against *P. infestans* in potato (Rosahl et al., 2006).

Abscisic acid (ABA) plays a major role in the regulation of plant water status, controls developmental processes such as seed germination and mediates abiotic stress tolerance (Christmann et al., 2006). Besides very sensitive mass spectrometry, these authors developed a non-invasive assay based on ABA-responsive luciferase expression. The primary sites of ABA action identified by *in planta* imaging corresponded to the sites of ABA biosynthesis, i.e., vasculature and guard cells. During the ABA response of guard cells, characteristic Ca^{2+} oscillations were observed and physiological and genetic evidence was provided that Ca^{2+}_{cyt} kinetics encode information that controls the magnitude of a graded physiological response. Signal pathways interfering with the ABA-induced Ca^{2+}_{cyt} kinetics either promote or reduce the ABA response. Cytoplasmic Ca^{2+} thus represents a level where the input from different signalling pathways is integrated to adjust stomatal responses to opposing demands. Mediators of the ABA-responsive gene expression include a number of transcriptional regulators of different types, such as the homeodomain protein AtHB6 which is a negative regulator of ABA responses. The complexity of ABA signal transduction which involves positive regulators and negative feedback loops possibly reflects intensive crosstalk with other signal pathways and the role of ABA as part of and integral to several responses (Christmann et al., 2006).

Despite decades of effort, today's understanding of auxin biosynthesis *in planta* is far from complete. Moreover, in the past few years it has become more and more obvious that regulation of plant growth and development is maintained by a high interconnection of different phytohormone functions rather than by the absolute amount of only one hormone. In this context Pollmann et al. (2006) dedicated their work to two major aspects. On the one hand, they established a highly sensitive GC-MS/MS method for the simultaneous analysis of ABA, IAA, jasmonic acid, oxophytodienoic acid, and salicylic acid. On the other hand, efforts were made to gain more insight into auxin biosynthesis in *Arabidopsis*. Here, these authors analyzed the physiological function of the nitrilase isoenzyme family, indicating an ancillary function of the nitrilases in plant basal auxin supply. Substantial evidence was provided that auxin is principally produced from L-tryptophan by a soluble enzyme complex, termed IAA synthase, in *Arabidopsis*. Additionally, indole-3-acetamide (IAM) was shown to be an endogenous compound in plants, and this substance can be converted *in planta* by a specific IAM hydrolase (AMI1) to indole-3-acetic acid, thus disclosing an alternative pathway of auxin synthesis, which was earlier considered to be exclusively active in plant pathogenic bacteria (Pollmann et al., 2006).

Maize contains relatively high concentrations of indole-3-acetic acid (IAA) conjugates in the endosperm. This allowed Kriechbaumer et al. (2006) to perform a series of incorporation experiments with ^{13}C-labelled precursors followed by NMR analysis. The pattern of ^{13}C nuclei incorporated into IAA after labelling with specific and general precursors demonstrated tryptophan dependency of IAA biosynthesis in this tissue. In the search for an IAA biosynthetic route from tryptophan, evidence for the importance of a pathway via indole-3-acetonitrile (IAN) was obtained. IAN is present in the kernel together with a nitrilase (ZmNIT2) that efficiently converts IAN to IAA (Kriechbaumer et al., 2006).

Indole-3-acetic acid (IAA) in low concentrations stimulates growth and development, while higher concentrations can be toxic to the plant. Therefore, tight control of IAA concentration is necessary for proper plant development. IAA conjugates are thought to be involved in the regulation of free IAA concentrations. Most studies done over the last fifty years showed that the amount of total IAA (free plus conjugated) obtained by direct hydrolysis of the tissue was much greater than the amount readily extractable with solvents. This discrepancy has only recently been resolved by the isolation of proteins and peptides to which IAA was attached (Seidel et al., 2006). In bean seeds, the majority of IAA is conjugated to large peptides and proteins. The gene for the major bean protein, *iap1,* which has IAA attached, was recently cloned. Constitutive expression of this gene resulted in an increased growth response in *Arabidopsis* while, under its native bean promoter, expression was exclusively found in seeds, indicating a specific role for this protein in auxin homeostasis (Seidel et al., 2006).

Christian et al. (2006) focus on the cellular site of auxin perception. They report on progress made in protoplast swelling assays, as well as on the auxin-insensitive *diageotropica* mutant of tomato, showing that the extracellular auxin binding protein (ABP1) perceives the signal. However, the *aux1* mutant and earlier work using auxin efflux inhibitors demonstrated that an important part of auxin perception occurs inside the cell, most probably via the recently identified auxin receptor TIR1. Methods were developed for recording rapid auxin-induced growth in the tiny *Arabidopsis* hypocotyls and flower stalk segments at high temporal resolution, the CCD auxanometer, as well as for detecting rapid auxin responses in protoplasts. Additionally, Christian et al. (2006) report on physiological evidence for a role of auxin-induced K^+ uptake channels in elongation growth.

This issue is further elaborated by Fuchs et al. (2006), who review the phytohormonal control of K^+ channels and their role in plant development. In *Arabidopsis,* auxin induction of K^+ channel transcripts is restricted to tissues displaying irreversible cell expansion upon the application of auxin, while the activity of these channels is not affected in cells undergoing reversible volume changes, such as guard cells. Auxin relocation in seedlings undergoing gravi- or phototropism induces the differential expression of the K^+ uptake channel gene *ZMK1.*

The spatial regulation of K^+ influx via potassium-selective channels thus seems to be a prerequisite for differential growth during tropic bending of maize coleoptiles, unravelling a link between auxin redistribution in photo- or gravistimulated plants and the molecular mechanisms leading to differential cell expansion and thus coleoptile bending. Stronger bending of maize seedlings photostimulated on a clinostat can be correlated to a steeper K^+ channel mRNA gradient in the opposing coleoptile halves, further providing evidence for the importance of K^+ channel regulation during differential growth (Fuchs et al., 2006).

Plant patterns have to integrate environmental cues and to cope with a high level of noise in the sensory outputs of individual cells, which can be achieved by systems composed of local self-amplification linked to lateral inhibition. Nick (2006) reports on auto-regulatory feedback loops in the context of auxin-triggered growth, focussing on actin responses to auxin in graminean coleoptiles and in tobacco cell cultures. He suggests that components relevant for auxin signalling are transported along actin microfilaments and that this transport is stimulated by auxin. Moreover, evidence is provided for crosstalk with jasmonate, which acts by downregulation of auxin responsiveness. By a combination of modelling and physiological manipulation, his group could demonstrate that auxin synchronizes the divisions of adjacent cells on the background of strong heterogeneity of individual cells. Nick (2006) concludes that self-amplification of auxin signalling, coupled to mutual competition of individual cells for available auxin, provide a versatile tool to fulfil the special requirements posed in patterning in plants.

Werner et al. (2006) review recent results on the role of cytokinin degradation in plants, which is catalyzed by cytokinin oxidase/dehydrogenase (CKX) enzymes. The authors have characterized the *CKX* gene family members of *Arabidopsis* and showed that differences in subcellular localization, expression pattern, and biochemical properties contribute to functional specification. Recent advances in understanding the enzymatic mechanism and the implications of a three-dimensional CKX enzyme structure are discussed. Importantly, CKX enzyme over-production generated, for the first time, cytokinin-deficient plants. Analysis of these plants revealed developmental processes which are regulated by cytokinin and thus contributed significantly to our understanding of cytokinin function. The exit of cells from the root meristem was recognized as a crucial growth regulatory event controlled by cytokinin. Furthermore, a recent breakthrough is highlighted in the article, namely the function of a *CKX* gene in yield control in rice, suggesting that CKX has the potential to become a valuable tool in plant biotechnology. Last but not least, the reported results corroborate the notion that fine-tuned control of cytokinin metabolism is as relevant as post-receptor signalling to determine cytokinin activity *in vivo* (Werner et al., 2006).

Mosses are interesting organisms for hormonal research as they represent early land plants and enable insights into an evolutionary early situation of developmental regulation. Von Schwartzenberg (2006) focusses on the metabolism and biosynthesis of cytokinins in the moss *Physcomitrella patens*. A comparison of metabolic differences with respect to seed plants is presented, indicating an important role of adenosine kinase for the formation of nucleotides during cytokinin interconversion. The importance of this interconversion for the regulation of cytokinin activity can also be deduced from the *ove* mutants of *Physcomitrella,* which are outstanding because of their enormous cytokinin over-production. Measurements of cytokinin interconversion revealed that the *ove* phenotype is correlated to an increased hydrolysis of cytokinin ribosides, resulting in release of the base. Further studies on cytokinin biosynthesis in moss will help to complete our understanding of the evolution of hormonal regulation (von Schwartzenberg, 2006).

Hartig and Beck (2006) focus on the crosstalk between auxin, cytokinin, and sugar during the cell cycle in a highly synchronized tobacco BY-2 cell culture. While the concentration of auxin did not show significant changes in the course of the cell cycle, the cytokinin signal showed marked oscillations, with concentration maxima prior to each transition from one phase of the cycle to the next, and minima close to zero in between. Any shift of the phases of these oscillations resulted finally in a delay of the cycle, even if the completion of an individual phase may be transitorily accelerated. The oscillations result from a cooperation of cytokinin synthesis and degradation, but not from cytokinin export. Cytokinin degradation is accomplished by cytokinin oxidase/dehydrogenase, whose activity depends on the levels of auxin and cytokinin. Proteins which regulate the progress of the cycle and whose expression responds differently to all three types of signals are the cyclins, especially of the A and the D type. Sucrose, administered to the dividing cells, has to be cleaved by invertase to become a signal for the cell cycle. Invertase activity, on the other hand, is under the control of auxin and cytokinin, demonstrating crosstalk of all three types of signals at the signal generation level. A similar example, however at the lowermost level, is shown by the cyclin-dependent kinase (CdkA), whose regulatory moieties – the cyclins – must be exchanged to trigger progression of the cell cycle. Auxin and cytokinin, in contrast to glucose and fructose, can be produced by the dividing cells. Their effective intracellular concentrations, however, depend in an auto-regulatory way on their external concentrations (Hartig and Beck, 2006).

How can all this ever-growing complexity be handled? Decker et al. (2006) describe their approaches to develop the moss *Physcomitrella* as a tool for plant systems biology. Simplicity of the tissue, with only about four different cell types, and specific cellular responses to auxin, cytokinin, and ABA make it ideal for such a holistic approach. Genomic, proteomic, and bioinformatic tools have been developed and numerous genes have been cloned and analyzed. Outstanding homologous recombination greatly facilitates gene functional analyses via reverse genetics approaches in *Physcomitrella.* A tight correlation between a unique cell cycle arrest and cell differentiation has been described, as well as a crosstalk between sugar and cytokinin integrated via the retinoblastoma pathway, a signalling cascade controlling progression through the cell cycle. Likewise, the integration of environmental signals to cellular differentiations via ABA has been elucidated in detail. Thus, moss systems biology is en route (Decker et al., 2006).

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