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Plant Hormones: The Interplay of Brassinosteroids and Auxin

Dispatch

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Interplay between hormones is crucial for the coordination of plant development. Recent work has provided important new insights into the close relationship between brassinosteroid and auxin signalling pathways in plants.

Systemic signalling molecules — 'hormones' — play key roles in establishing the developmental program in plants; they are also intimately involved in shaping plant growth and development in response to environmental cues such as light [1]. First identified in 1979 by Grove *et al.* [2], brassinosteroids are the most recently characterised group of plant hormones and are fast emerging as influential growth regulators. Brassinosteroids control a broad range of responses in plants, including seed germination, stem and root elongation, vascular differentiation, leaf expansion and apical dominance. Interestingly, each of these responses is also controlled by a second hormone, auxin, suggesting there might be considerable interplay between these two hormones in the control of development.

Although there is evidence that brassinosteroids and auxin can operate independently [3–7], other studies have demonstrated a link between brassinosteroids and auxin, indicating that some pathways are under dual control. For example, brassinosteroids and auxin have been shown to act synergistically in the control of hypocotyl elongation in a variety of species [8]. The mechanisms by which these two hormones are linked have eluded us until recently. Nemhauser *et al.* [9] have now reported evidence that brassinosteroid and auxin signalling pathways converge at the level of the transcriptional regulation of common target genes.

Molecular genetic approaches are gradually revealing the nature of brassinosteroid signal transduction. Several components in the brassinosteroid biosynthesis and signalling pathways have been established through the identification of mutants: for example, *det2*, *cpd*, *dwf1*, *bas1* and *sax1*, where brassinosteroid biosynthesis is affected, and *bri1*, *bin2*, *bes1*, *bzr1* and *bak1*, where signalling is affected [9,10]. Brassinosteroids are perceived by the BRI1 receptor kinase, which is located in the cell membrane and is thought to act in concert with BAK1, a second receptor kinase. The brassinosteroid signal is transduced via nuclear-localised proteins BZR1 and BES1. BIN2, a negative regulator of the pathway, controls this process by phosphorylating BZR1 and BES1, which are then degraded by the proteosome. Brassinosteroids appear to control the pathway, at least in part, by negatively regulating BIN2 and consequently relieving the repression of BZR1 and BES1 activity.

In recent years, significant progress has been made in elucidating the pathway of auxin signal transduction. In an emerging model, auxin affects gene expression, and thus developmental outcomes, by controlling the abundance of AUX/IAA family transcriptional repressor proteins [11]. AUX/IAAs are thought to regulate transcription by heterodimerising with, and thereby altering the activity of, auxin response factors (ARFs) [11]. ARFs modulate transcription by binding to conserved auxin response elements in the promoters of auxin-regulated genes, including those encoding the AUX/IAAs.

The balance of AUX/IAAs and ARFs is thus a key control point in auxin signalling, and this is reflected in its dynamic properties. Auxin alters this balance by stimulating the ubiquitin-mediated proteolysis of AUX/IAA proteins via the ubiquitin ligase SCF^{TIR1}, and this feeds back to regulate AUX/IAA transcription. The importance of this mechanism is evident from genetic analysis. Mutations that stabilise AUX/IAAs, such as *axr2-1* or *axr3-1*, or mutations in components of the SCF^{TIR1} ubiquitination machinery, such as *tir1-1* or *axr1-12*, cause a range of auxin related phenotypes including resistance to topically applied auxin [11].

Crosstalk between hormones is essential for the coordination of development throughout a plant's life cycle [1]. Unravelling these interwoven pathways will be important for understanding the network that underpins plant development. Recent work has taken us forward in this process, highlighting a significant overlap in brassinosteroid and auxin signalling [7,9]. It was not obvious that strong connections would be found between these two hormones, given their respective kinetics of action. In soybean, for example, brassinolide, the most active brassinosteroid, was found to stimulate epicotyl elongation 45 minutes after application, gradually rising to a peak after several hours [3]. This contrasts with the relatively rapid auxin response, which commences after 10–15 minutes and peaks at 30–45 minutes. Similar kinetics have also been reported at the level of gene regulation in *Arabidopsis* [5]. But taking a multifaceted approach, Nemhauser *et al.* [9] have now demonstrated that there are strong links between brassinosteroid and auxin control of tissue elongation and gene regulation.

Nemhauser *et al.* [9] used *Arabidopsis* mutants with perturbed brassinosteroid or auxin signalling to demonstrate the interdependence of these two hormones in the control of hypocotyl elongation. Seedlings were grown on a range of brassinolide concentrations at 22°C or 26°C. Growth at the higher temperature had previously been shown to enhance auxin levels, which correlated with increased elongation growth [12]. These experiments showed

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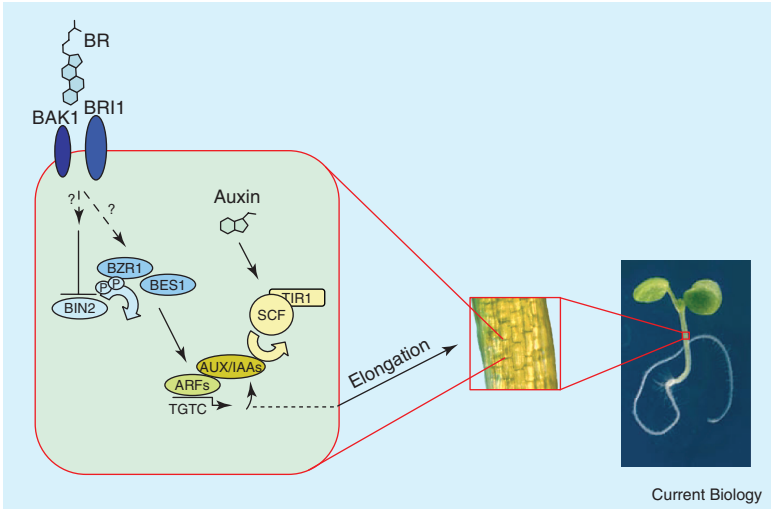

Figure 1. Brassinosteroid and auxin signalling in *Arabidopsis*.

Brassinosteroid signalling: brassinosteroid perception by the BRI1–BAK1 receptor complex is followed by dephosphorylation and nuclear localisation of BZR1 and BES1, resulting in the regulation of target genes. BIN2 phosphorylates BZR1 and BES1, leading to their degradation by the proteosome. This process is negatively regulated by brassinosteroids. Auxin signalling: auxin controls signal transduction via the ubiquitin ligase SCF^{TIR}. AUX/IAAs bind to ARF transcriptional regulators to block their action. In the presence of auxin, AUX/IAA proteins interact with TIR1 and are degraded by the proteosome. This relieves the repressive effects of AUX/IAAs on ARF action and transcriptional regulation can resume. Brassinosteroid and auxin crosstalk: the brassinosteroid and auxin signalling pathways converge at the level of transcriptional regulation of target genes with common regulatory elements.

that brassinosteroids interact synergistically with temperature-regulated hypocotyl elongation. Furthermore, intact brassinosteroid and auxin pathways were both required for this response. These data suggest a strong interdependence between brassinosteroid and auxin signalling in the control of hypocotyl elongation in *Arabidopsis*. Nemhauser *et al.* [9] have also found that the brassinosteroid receptor *bri* mutation can substantially suppress the elongated *yucca* mutant phenotype, which is caused by elevated auxin levels. This suggests that brassinosteroids are required for the *yucca* phenotype, strengthening the hypothesis.

Synergistic interactions between brassinosteroids and auxins have been reported previously by Mandava *et al.* [8] and more recently, in the control of lateral root growth, by Bao *et al.* [13]. Furthermore, the auxin-insensitive mutant *axr1-3* was also shown to be brassinolide-insensitive for shoot elongation [14]. In contrast, however, the brassinosteroid mutants *bri* and *sax1* showed enhanced inhibition of root growth in response to exogenous auxin [15,16]. So it appears that brassinosteroid and auxin crosstalk is important in the control of elongation growth, but genetic and physiological analysis suggests their mode of interaction may be distinct in different tissues and possibly species-specific.

Having established a firm link between brassinosteroid and auxin signalling, the next step was to identify precisely where and how the pathways intersect. Several earlier studies (for example [6,7]) identified auxin-responsive genes as common targets for transcriptional regulation by brassinosteroids. This work also identified a handful of genes that were brassinosteroid-regulated after a relatively short lag period, providing the possibility for synchronised brassinosteroid and auxin action in the regulation of specific genes. To extend this analysis Nemhauser *et al.* [9] used 22K Affymetrix chips to identify genes with differential expression in brassinolide *versus* mock treated seedlings. The results were compared with auxin-regulated genes identified in a similar fashion by Zhao *et al.* [17]. Of the 342 and 336 genes found to be upregulated by brassinolide and auxin, respectively, a quarter were regulated by both hormones within a similar (early) time period. These observations indicate that, although brassinosteroid and auxin regulate a large number of genes independently, a substantial subset appear to be common targets for both hormones.

These results are comparable to those obtained by Goda *et al.* [7] using the 8K Affymetrix chip, though in that study fewer shared target genes were identified, which probably reflects differences in the size of the Affymetrix chip, genotypes, growth conditions and analysis techniques used in the two laboratories. Furthermore, different concentrations of brassinolide were used in the two experiments, known to be an important factor in the brassinolide-regulation of specific genes [18,19]. In line with previous reports, both studies [7,9] identified genes in the *AUX/IAA*, *SAUR* and *GH3* families as dual targets for brassinosteroid and auxin.

Nemhauser *et al.* [9] went on to show that the TGTCTC auxin-responsive element is enriched in the brassinolide-regulated gene promoter sequences, whilst the TGTC core sequence is enriched in brassinolide, auxin and dual-regulated genes. These findings concur with those of Goda *et al.* [7], suggesting that the brassinolide and auxin signals converge at ARFs to regulate transcription (Figure 1). Indeed, a reporter fusion gene *DR5::GUS*, which contains a highly responsive synthetic auxin response element, has recently been shown to be under the control of brassinolide [9,13,18]. Another common consensus sequence in these genes is the MYC/bHLH element, suggesting another level of dual control.

One of the major problems in establishing a link between brassinosteroid and auxin signalling has been squaring the different kinetics of brassinosteroid and auxin-induced responses (see above). Nakamura

et al. [14] have gone some way toward addressing this discrepancy: they have shown that *SAUR-AC1* is regulated synergistically by brassinosteroid and auxin as early as 15 minutes post hormone exposure. At this time point, neither hormone applied alone has a significant effect on *SAUR-AC1* expression. This means that genes thought previously to be regulated independently may be under dual control.

Nemhauser *et al.* [9] also demonstrated synergistic interaction of brassinosteroid and auxin in the case of four shared target genes selected from their microarray data. But the complexity of brassinosteroid and auxin signalling does not stop there. Brassinosteroids have been shown to alter auxin levels: for example, the *det2* mutant was found to have higher levels of auxin, and brassinosteroids appear to regulate *NIT3*, a gene involved in auxin biosynthesis [18,20]. This is not the means via which brassinosteroid regulates transcription, as the increased auxin levels in the *det2* mutant are at odds with its impaired auxin responsiveness. Furthermore, topically applied brassinolide has no effect on endogenous auxin levels [18].

Brassinosteroids have been shown to regulate the expression of *PIN* genes, which encode essential components in the polar transport of auxin [7,9,19]. Similarly, auxin may have a role in the control of *BRI*, *BRL2* and *BRL3* expression, and may therefore influence brassinosteroid signalling capacity [9]. These studies suggest that the interactions between brassinosteroid and auxin are extensive and complex.

The interplay between different signalling pathways is crucial for plant development. The external environment has a constant impact on this process manipulating development to a large extent through the hormonal pathways [1]. Coordination of the multiple external signals with the developmental program is achieved through crosstalk providing the flexibility of response required to maximise reproductive success in environments that can be subject to frequent change. The major challenge for the future will be to establish how multiple pathways are coordinated and to determine the fundamental principles that underpin network interplay.

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