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Brassinosteroid signalling

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Summary

The brassinosteroid (BR) class of steroid hormones regulates plant development and physiology. The BR signal is transduced by a receptor kinase-mediated signal transduction pathway, which is distinct from animal steroid signalling systems. Recent studies have fully connected the BR signal transduction chain and have identified thousands of BR target genes, linking BR signalling to numerous cellular processes. Molecular links between BR and several other signalling pathways have also been identified. Here, we provide an overview of the highly integrated BR signalling network and explain how this steroid hormone functions as a master regulator of plant growth, development and metabolism.

Key words: Brassinosteroid, Gibberellin, Photomorphogenesis, Receptor kinase

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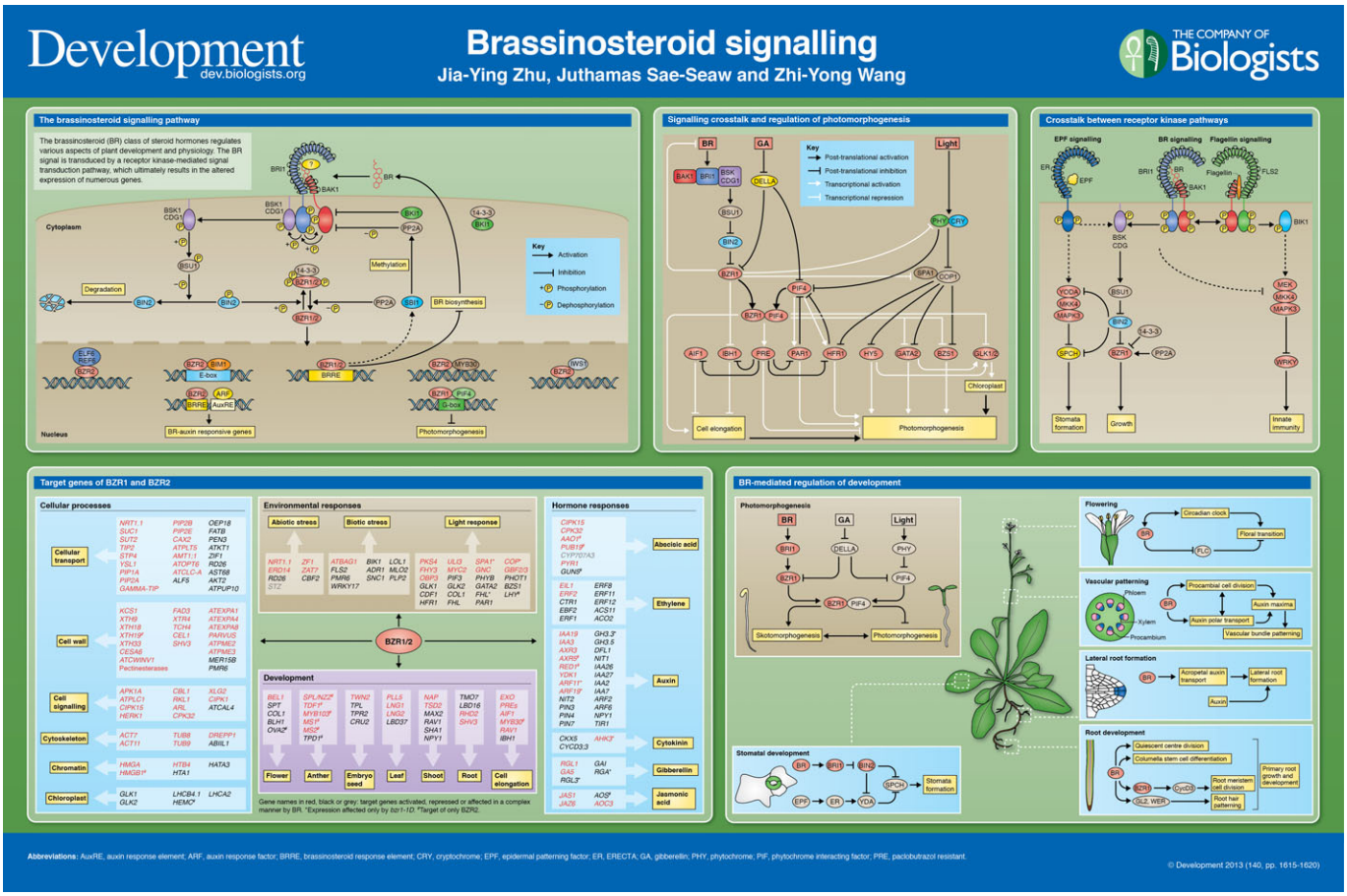
Introduction

Steroid hormones regulate gene expression and development in both plants and animals. Brassinosteroid (BR) was discovered in pollen extract based on its ability to promote cell elongation, but was later found in all growing tissues of higher plants, with the highest levels found in pollen, seeds and fruit. Subsequent physiological studies demonstrated that BR has a wide range of effects on growth and in responses to biotic and abiotic stresses. Studies of mutants with defects in BR biosynthesis or signalling demonstrated that BR plays essential roles in nearly all phases of plant development, as these mutants show multiple developmental defects, such as reduced seed germination, extreme dwarfism, photomorphogenesis in the dark, altered distribution of stomata, delayed flowering and male sterility. The molecular mechanisms of BR signal transduction and BR-mediated regulation of gene expression and plant development have been dissected in detail by studies using molecular genetics, biochemistry, proteomics and genomics, making the BR signalling pathway one of the best understood signal transduction pathways in plants.



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BR signal transduction: from receptor kinases to transcription factors

BR binds to the extracellular domain of a cell-surface receptor kinase, BRASSINOSTEROID INSENSITIVE1 (BRI1), to activate a signal transduction cascade that regulates transcription (Clouse, 2011; Kim and Wang, 2010; Wang et al., 2012). BR binding activates BRI1 kinase activity (Hothorn et al., 2011; Kinoshita et al., 2005; Li and Chory, 1997; She et al., 2011; Wang et al., 2001). BRI1 activation involves recruitment of the co-receptor kinase BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) (Gou et al., 2012; Li et al., 2002; Nam and Li, 2002), disassociation of the inhibitory protein BRI1 KINASE INHIBITOR1 (BKI1) (Jaillais et al., 2011; Wang et al., 2011; Wang and Chory, 2006) and sequential transphosphorylation between the kinase domains of BRI1 and BAK1 (Clouse, 2011; Wang et al., 2008). Phosphorylated BKI1 also interacts with the 14-3-3 family of phosphopeptide-binding proteins to promote BR signalling (Wang et al., 2011). BRI1 and BAK1 are mainly localised at the plasma membrane but also undergo endocytosis and cycle between the endosome and plasma membrane (Geldner et al., 2007; Irani et al., 2012; Russinova et al., 2004; Song et al., 2009a).

Upon BR activation, BRI1 then phosphorylates members of two groups of plasma membrane-anchored cytoplasmic kinases represented by BRASSINOSTEROID-SIGNALLING KINASE1 (BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH1 (CDG1) (Kim et al., 2011; Tang et al., 2008). This phosphorylation promotes BSK1 and CDG1 binding to, and phosphorylation of, the phosphatase BRI1-SUPPRESSOR1 (BSU1) (Kim et al., 2011; Kim et al., 2009; Mora-García et al., 2004). BSU1 inactivates the GSK3-like kinase BRASSINOSTEROID INSENSITIVE2 (BIN2) by dephosphorylating a conserved tyrosine residue (Kim and Wang, 2010). In the absence of BR, or when BR levels are low, active BIN2 phosphorylates two homologous transcription factors, BRASSINAZOLE RESISTANT1 (BZR1) and BZR2 [also named BRI1-EMS-SUPPRESSOR1 (BES1)] (He et al., 2002; Wang et al., 2002; Yin et al., 2002), to abolish their DNA-binding activity and cause their cytoplasmic retention by 14-3-3 proteins (Bai et al., 2007; Gampala et al., 2007; Vert and Chory, 2006). When BR levels are high, BIN2 is inactivated by BSU1 and degraded by the proteasome (Peng et al., 2008), and BZR1 and BZR2 are dephosphorylated by PROTEIN PHOSPHATASE 2A (PP2A) (Tang et al., 2011). Unphosphorylated BZR1 and BZR2 can then move into the nucleus and bind to the promoters of their target genes, leading to gene activation or repression (He et al., 2005; Sun et al., 2010; Yin et al., 2005; Yu et al., 2011).

BZR1 and BZR2 transcription factors share 88% overall sequence identity and 97% identity in their DNA-binding domains (Wang et al., 2002). Early studies showed that BZR1 binds to the BR-response element (BRRE, CGTG$^{C}/_{T}$G) and represses gene expression (He et al., 2005), whereas BZR2 interacts with a basic helix-loop-helix (bHLH) factor [BES1 INTERACTING MYC-LIKE1 (BIM1)] and together they bind to the E-box (CANNTG) element and activate gene expression (He et al., 2005; Yin et al., 2005). However, recent studies have shown that BZR1 and BZR2 have similar DNA-binding specificities and transcription activities (Sun et al., 2010; Yu et al., 2011).

Target genes of BR signalling

Genome-wide protein-DNA interaction analyses combined with expression profiling have identified several thousand *in vivo* binding targets of BZR1, including more than 1000 BR-regulated BZR1 target genes (Sun et al., 2010). A smaller set of targets, which overlaps significantly with the BZR1 targets, has been identified for BZR2 (Gudesblat and Russinova, 2011; Yu et al., 2011). These BZR1/BZR2 target genes reveal numerous molecular links to specific cellular, metabolic and developmental processes. In particular, cell wall modification and cellular transport are major cellular functions targeted by BR, consistent with its effects on cell elongation and growth (Sun et al., 2010). Interestingly, a recent study showed that compromised cell wall integrity activates BR signalling, suggesting a feedback mechanism for the BR-mediated balance between cell extension and integrity of the cell wall (Wolf et al., 2012). Also highly represented in the BZR1 targets are transcription factors and components of many other signalling pathways, such as the light, gibberellin (GA) and auxin pathways (Sun et al., 2010).

Interestingly, BZR1 and BZR2 also feedback and control the expression of genes encoding BR biosynthetic enzymes and upstream BR signalling components (He et al., 2005; Sun et al., 2010; Yu et al., 2011). Furthermore, BR also induces the expression of the SUPPRESSOR OF BRI1 (SBI1) leucine carboxylmethyltransferase, which methylates PP2A and promotes PP2A localisation to membranes, where it dephosphorylates and inactivates the internalised BRI1, providing another mechanism of feedback regulation (Wu et al., 2011).

BR-responsive gene expression is mediated by BZR1 and BZR2 as well as additional interacting transcription factors. In addition to BIM1 (Yin et al., 2005), BZR2 interacts with the transcription factors MYB30 (Li et al., 2009; Yin et al., 2005), INTERACTS WITH SPT6 1 (IWS1) (Li et al., 2010b), EARLY FLOWERING6 (ELF6) and the histone H3 lysine 27 demethylase RELATIVE OF ELF6 (REF6) (Lu et al., 2011; Yu et al., 2008). Genetic and transgenic experiments indicated that these BZR2-interacting proteins have only minor effects on BR-regulated growth responses such as hypocotyl elongation; whether they interact with BZR1 remains unknown. Both BZR1 and BZR2 interact with the phytochrome-interacting factor (PIF) family of bHLH factors and the GA signalling DELLA proteins to co-regulate the expression of a large number of genes, cell elongation and photomorphogenesis (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012; Oh et al., 2012).

BR-mediated regulation of photomorphogenesis through crosstalk with other pathways

Nearly all BR-regulated processes are also regulated by other hormonal or environmental signals (Depuydt and Hardtke, 2011). In particular, suppression of photomorphogenesis in the dark requires BR and two other hormones: GA and auxin (Li et al., 1996; Szekeres et al., 1996; Lau and Deng, 2010). A large body of evidence shows that BR antagonises light signals, has similar physiological effects to GA, and interacts synergistically with auxin during cell elongation and gene expression (Depuydt and Hardtke, 2011; Lau and Deng, 2010). Recent studies have uncovered the molecular mechanisms underlying the interactions between BR and these other signalling pathways (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012; Luo et al., 2010; Oh et al., 2012).

Regulation of the light response

Light switches seedling development from etiolation (skotomorphogenesis) to de-etiolation (photomorphogenesis) by inhibiting cell elongation and promoting chloroplast development and leaf expansion. BR is required not only for cell elongation but also for the suppression of light-induced genes in the dark (Chory et al., 1991; Clouse et al., 1996; Li et al., 1996; Song et al., 2009b; Sun et al., 2010; Szekeres et al., 1996). Although it is generally

believed that environmental signals affect plant growth by changing plant hormones, light has no obvious effect on BR levels or upstream BR signalling (Luo et al., 2010; Symons et al., 2008). Instead, BR, acting through BZR1, appears to modulate light signalling at multiple levels (Wang et al., 2012). First, BZR1 represses the expression levels of many positive regulators and activates negative regulators of the light signalling network (Fan et al., 2012; Luo et al., 2010; Sun et al., 2010). BZR1 also directly interacts with PIFs to co-regulate a large number of light- and BR-responsive target genes (Oh et al., 2012). PIFs are negative regulators of photomorphogenesis and are degraded upon interaction with light-activated phytochromes, but are increased by dark and shade, as well as heat (Leivar and Quail, 2011). PIFs and BZR1 directly interact with each other and interdependently regulate a large number of direct target genes, many of which encode transcription factors and proteins that function in the cell wall and chloroplast (Oh et al., 2012).

Some of the transcription factors downstream of BZR1 play important roles in regulating cell elongation and chloroplast development. For example, promotion of cell elongation by BZR1 and PIFs requires activation of the PACLOBUTRAZOL-RESISTANCE (PRE) family of HLH factors (Bai et al., 2012; Oh et al., 2012). PREs interact with and inhibit several other HLH/bHLH factors, including ILI1 BINDING bHLH1 (IBH1) (Zhang et al., 2009), ATBS1-INTERACTING FACTOR1 (AIF1) (Wang et al., 2009), PHYTOCHROME RAPIDLY REGULATED1 (PAR1) (Hao et al., 2012) and LONG HYPOCOTYL IN FAR-RED1 (HFR1) (Hyun and Lee, 2006). PAR1 and HFR1 bind to and inhibit PIF4 (Hao et al., 2012; Hornitschek et al., 2009), and therefore BR activation of PREs would lead to inhibition of PAR1 and HFR1 and to an increase in PIF4 activity (Hao et al., 2012). BR-mediated inhibition of chloroplast development is correlated with repression of the two GOLDEN 2-LIKE (GLK) transcription factors (GLK1 and GLK2), which are major regulators of nuclear genes encoding chloroplast proteins (Sun et al., 2010; Waters et al., 2009; Yu et al., 2011), as well as of the nuclear-encoded chloroplast protein BRASSINAZOLE INSENSITIVE PALE GREEN2 (BPG2), which is required for the translation of chloroplast-encoded photosynthetic proteins (Komatsu et al., 2010).

The relationship between BR and GA

BR and GA are both growth-promoting hormones, having similar effects on various developmental processes throughout the life cycle of plants (Depuydt and Hardtke, 2011). GA binds to its receptor GIBBERELLIN INSENSITIVE DWARF1 (GID1) to induce ubiquitylation and degradation of DELLA family proteins, which bind to several transcription factors, including PIFs, to inhibit their DNA binding. GA induces degradation of DELLA to derepress PIFs (de Lucas et al., 2008; Feng et al., 2008). Until recently, the effects of BR and GA have been considered additive (Depuydt and Hardtke, 2011), and their signalling pathways have been proposed to act on largely non-overlapping transcriptional responses (Nemhauser et al., 2006). However, recent studies demonstrated an interdependent relationship and a direct interaction between the BR and GA signalling pathways (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012). GA is unable to increase hypocotyl elongation in BR-deficient and BR-insensitive mutants, whereas BR and the dominant gain-of-function *bzr1-1D* mutation can increase cell elongation in GA-deficient mutants. Furthermore, DELLA proteins directly interact with BZR1 and inhibit its DNA binding, and thus GA regulates gene expression by releasing DELLA inhibition of BZR1 (Bai et al., 2012; Gallego-Bartolomé et al., 2012; Li et al., 2012). Together, these studies demonstrate that direct interactions among BZR1, PIFs and DELLA proteins integrate BR, light and GA signals and regulate a central transcriptome that programs photomorphogenesis (Bai et al., 2012; de Lucas et al., 2008; Feng et al., 2008).

BR interactions with auxin signalling

Microarray analyses showed that BR and auxin also activate overlapping sets of genes, while genetic and physiological studies showed synergistic and interdependent interactions between the two hormones in a wide range of developmental contexts, such as hypocotyl elongation and vascular bundle patterning (Depuydt and Hardtke, 2011; Ibañes et al., 2009; Nemhauser et al., 2004). Several observations suggest potential molecular mechanisms of mediating BR-auxin interactions. First, BZR1 regulates many genes involved in auxin synthesis, transport and signalling (Sun et al., 2010). Second, auxin activates BR biosynthetic genes and increases BR levels (Chung et al., 2011; Mouchel et al., 2006; Yoshimitsu et al., 2011). Third, BIN2 phosphorylates an auxin-response factor (ARF2) (Vert et al., 2008). Fourth, BZR2 and ARF5 bind to the same BR/auxin-response promoter [*SMALL AUXIN UPREGULATED15* (*SAUR15*)] (Walcher and Nemhauser, 2012). Fifth, BR and auxin responses are integrated through the actin cytoskeleton, which is not only regulated by both BR and auxin but also feedback regulates auxin transport and BR signalling (Lanza et al., 2012). The contributions of these mechanisms to the physiology of BR-auxin interaction remain to be evaluated further.

Crosstalk between BRI1 and other receptor kinase pathways

There are over 400 receptor kinases in *Arabidopsis* (Shiu et al., 2004). Among them, the BR/BRI1 signalling pathway remains the best characterised. Sharing and cross-regulation of downstream components are thus likely to be common among receptor kinase pathways, particularly those that diverged recently in evolution and those that regulate related processes and thus would benefit from signalling crosstalk. Indeed, recent studies have shown that direct crosstalk exists between BRI1 and two other well-characterised receptor kinases that control stomata development and innate immunity.

Crosstalk between the BRI1 and ERECTA pathways regulates stomata development

Photosynthesis requires both chloroplasts and stomata, which are the epidermal valves that allow gas exchange between plant leaves and the atmosphere. The density and distribution of stomata are tightly controlled to optimise the uptake of CO_2 and minimise water loss (Dong and Bergmann, 2010). The genetically defined stomata pathway includes EPIDERMAL PATTERNING FACTOR (EPF) family peptide signals, members of the ERECTA family of receptor kinases, the mitogen-activated protein kinase (MAPK) module and several bHLH transcription factors (Dong and Bergmann, 2010). BR has been shown to inhibit and promote stomatal development in leaves and hypocotyls, respectively, due to BIN2-mediated phosphorylation of two components of the stomatal pathway. Loss of BSU1 family phosphatases causes massive stomata formation, and this phenotype requires BIN2 activity (Kim et al., 2012). BIN2 phosphorylates and inactivates the MAPK kinase kinase YODA (YDA), which inhibits stomata formation. BR inactivation of BIN2 leads to activation of MAPKs and hence inhibition of stomatal development in leaves. Genetic evidence suggests that members of the BSU1 and GSK3 families might also mediate ERECTA signalling (Kim et al., 2012). BR also positively affects stomatal

development in hypocotyls through BIN2 phosphorylation and inactivation of the bHLH factor SPEECHLESS (SPCH) (Gudesblat et al., 2012), which acts downstream of the MAPKs and promotes cell division and stomata formation (Dong and Bergmann, 2010). It thus seems that multiple connections between the BRI1 and ERECTA receptor kinase pathways mediate the complex regulation of stomatal development.

Crosstalk between BRI1 and the FLS2 receptor kinase pathway regulates innate immunity

BAK1 functions as a co-receptor kinase not only for BRI1 but also for FLAGELLIN-SENSITIVE2 (FLS2), the receptor kinase that perceives bacterial flagellin signal and induces innate immunity (Chinchilla et al., 2009). It has been proposed that such co-receptor sharing could create an antagonistic relationship between BR and flagellin due to competition for the co-receptor (Belkhadir et al., 2012), although competition might only be significant when the amount of co-receptor is limiting (Wang, 2012). If the activated BAK1 can reassociate with different partners, activation of BAK1 by one signal might enhance the signalling of another pathway. However, there is evidence that BR can also inhibit FLS2-mediated signalling through an unknown mechanism downstream of the receptor kinases (Albrecht et al., 2012). Finally, it has been shown that BR represses *FLS2* expression through BZR1 and also regulates the MAPKs involved in FLS2 signalling and innate immunity (Kim et al., 2012; Sun et al., 2010). The contributions of these mechanisms to the trade-off between growth and immunity remain to be analysed.

BR-mediated regulation of other aspects of plant development

In addition to direct crosstalk with other signalling pathways, BR also impinges on developmental pathways, often through BZR1-mediated regulation of specific developmental regulators. Recent studies have shed light on BR regulation of reproductive development and root growth.

Regulation of reproductive development

The transition from vegetative to reproductive growth is regulated by numerous interacting endogenous and environmental cues, such as GA, BR, photoperiod and temperature (Li et al., 2010a). BR-deficient or BR-insensitive mutants in *Arabidopsis* exhibit late-flowering phenotypes, which is at least partly due to increased expression of the floral repressor *FLOWERING LOCUS C* (*FLC*) (Domagalska et al., 2007). *FLC* expression is negatively regulated by REF6 (Noh et al., 2004), and REF6 was shown to interact with BZR2 (Yu et al., 2008). However, whether this interaction mediates BR repression of *FLC* expression and promotion of flowering remains unanswered (Clouse, 2011), as REF6 activates gene expression by removing repressive H3K27me3 histone marks (Lu et al., 2011). BR may also indirectly affect flowering time by influencing the circadian clock and the photoperiod flowering pathway, as BR application shortens circadian rhythms (Hanano et al., 2006).

Defects in BR biosynthesis or signalling pathways also reduce male fertility due to shortening of the stamen and defects in pollen development (Ye et al., 2010). These developmental defects correlate with the reduced expression of several key genes involved in anther and pollen development, many of which are transcriptional targets of BZR2 (Ye et al., 2010). Finally, recent studies have shown that, in maize, BR controls sex determination by promoting stamen and repressing pistil development in tassels (Hartwig et al., 2011; Makarevitch et al., 2012).

Regulation of root development

Although BR is generally known to promote growth, the effect of BR on root growth is dose dependent (Müssig et al., 2003). Recent studies have shown that balanced actions of BR on cell division and differentiation are required for optimal root growth and meristem homeostasis (González-García et al., 2011; Hacham et al., 2011). BR promotes mitotic activity and expression of CYCLIN D3 and CYCLIN B1 in the root meristem (González-García et al., 2011; Hacham et al., 2011). However, high concentrations of BR accelerate cell differentiation/elongation and reduce meristem size and root growth. BR also promotes quiescent centre division and columella stem cell differentiation (Gonzalez-Garcia et al., 2011). Interestingly, expressing *BRI1* using an epidermal-specific promoter rescued the root growth of the *bri1* mutant, demonstrating that BR perception in the epidermis is sufficient to control root growth and meristem size, possibly through a mobile factor other than BZR1 or BZR2 (Hacham et al., 2011).

BR also plays an important role in directing epidermal cell fate in roots, where epidermal cells differentiate into hair or non-hair cells depending on their position. Blocking BR signalling disrupts the patterning of root hair cells and the expression patterns of *WEREWOLF* (*WER*) and *GLABRA2* (*GL2*), which specify epidermal cell fate (Kuppusamy et al., 2009). Thus, regulating the expression levels of cell type-specific components seems to be a general mechanism by which BR exerts specific effects on diverse developmental responses.

Perspectives

Over the past decade, our understanding of the BR signalling pathway has progressed rapidly thanks to a combination of genetic, proteomic and genomic approaches. The BR pathway represents the first, and still the only, fully elucidated receptor kinase signalling pathway in plants. Its extensive molecular connections with other signalling pathways demonstrate a high degree of integration in the regulatory networks in plants. In particular, the essential roles played by BR in plant responses to light and GA support the notion that BR is a master regulator at the centre of the plant growth regulation network. A framework has thus been established for building a detailed molecular map of the growth regulation network in plants.

Many key questions remain to be answered, however. For example, what controls the wide range of BR levels found in different tissues and organs? How do plants use BR in the context of normal development and under environmental stresses? Does BR serve as a positional cue for cell differentiation and morphogenesis? How does BR induce distinct responses in different tissues and cell types? How is the BR pathway integrated with additional signalling pathways? Finally, how does BR signalling integrate with (both by regulating and being regulated by) cellular processes such as vesicle trafficking, cytoskeleton organisation, and cell wall expansion and integrity? Answers to these questions will advance our understanding of plant growth regulation, which is important for food and bioenergy production and for environmental conservation (Vriet et al., 2012).

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Competing interests statement
The authors declare no competing financial interests.

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