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Review

Brassinosteroids in growth control: How, when and where

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ABSTRACT

The steroid hormones brassinosteroids take on critical roles during various plant growth processes, including control of cell proliferation and cell elongation. In this review, we discuss different strategies that have advanced our understanding of brassinosteroid function. Approaches observing whole-plant responses uncovered regulatory brassinosteroids-dependent modules controlling cell elongation. In these regulatory modules, downstream components of the brassinosteroid signaling pathway directly interact with other hormonal and environmental pathways. In alternative approaches, brassinosteroid activity has been dissected at the tissue and cellular level of above- and below-ground organs. These studies have determined the importance of brassinosteroids in cell cycle progression and in timing of cell differentiation. In addition, they have demonstrated that local reduction of the hormone sets organ boundaries. Finally, these studies uncovered the capacity of the epidermal-derived brassinosteroid signaling to control organ growth. Thus, inter-cellular communication is intimately involved in brassinosteroid-mediated growth control. The current challenge is therefore to decipher the spatiotemporal distribution of brassinosteroid activity and its impact on coherent growth and development.

Contents

1. Introduction

Brassinosteroids make up a group of plant steroid hormones that was originally isolated from pollen as growth-promoting substances. Brassinosteroids are present in young tissues but their precise spatial and subcellular distribution remains unknown. The two known active brassinosteroid forms are brassinolide and its immediate precursor castasterone, whose complete biosynthesis pathways are reviewed in [1,2]. The essential role of brassinosteroids in regulating plant growth and development was recognized two decades ago, when mutants deficient in either brassinosteroid biosynthesis or perception were first identified [3–6]. Their unique phenotype (first documented in [7,8]) includes a wide range of abnormalities, such as dwarfism, reduced male fertility, delayed flowering time, altered vascular development and impaired photomorphogenesis. The unequivocal importance of the hormone thus stimulated a seemingly simple universal question: how do brassinosteroids regulate growth? In this review, we outline the evolution of brassinosteroid research, discuss the contribution of whole-plant-based and the more recent tissue-specific-based approaches and highlight a recently reported mechanism controlling spatial distribution of brassinosteroids. We primarily focus on studies where Arabidopsis hypocotyl length and root growth served as experimental systems to infer brassinosteroid-mediated control of cell elongation and cell division in plants, respectively. Studies using other plant species and experimental systems that further contributed to our

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understanding of these two growth processes are also highlighted. Finally, we delineate future challenges in defining the mechanistic pathway regulating brassinosteroid function.

2. Zooming out: the canonical brassinosteroid signaling pathway

Substantial efforts, combining both genetics and biochemical approaches, have revealed the complete sequence of molecular events necessary for transmitting the brassinosteroid signal from the cell surface to the nucleus. Here, we will touch upon key elements in the canonical brassinosteroid signaling pathway in flowering plants (first identified in *Arabidopsis*), while readers interested in a detailed description of the brassinosteroid signaling cascade are referred to recent reviews [9–13]. Brassinosteroids are perceived at the cell surface, where they directly bind the extracellular leucine-rich repeat (LRR) module of BRASSINOSTEROID INSENSITIVE1 (BRI1), an LRR-receptor kinase [14–16]. However, the mode by which brassinosteroids reach the cell surface is still unknown. From the plasma membrane, the signal is transmitted to the nucleus, in a multi-step process involving the degradation of the GSK3-like kinase BRASSINOSTEROID INSENSITIVE2 (BIN2), the key inhibitor of the pathway [17–19]. When brassinosteroid levels are low, BIN2 levels rise, leading to phosphorylation and consequential inhibition of the downstream homologous transcription factors BRASSINAZOLE RESISTANT1 (BZR1) and BRI1-EMS-SUPPRESSOR1 (BES1)/BZR2 [20–22]. When brassinosteroids levels are high, a conformational change in BRI1 leads to a series of autophosphorylation and trans-phosphorylation events that fully activate the receptor and eventually trigger the proteasomal degradation of BIN2 [23]. This enables the subsequent dephosphorylation of BZR1 and BES1/BZR2 by a PROTEIN PHOSPHATASE 2A (PP2A) [24], allowing them to accumulate in the nucleus, where they homo- or heterodimerize and finally bind DNA at defined known *cis*-elements and regulate the expression of many genes, the nature of which will be elaborated below [25–28]. BZR1 and BES1/BZR2 are plant-specific, highly homologous transcription factors that bear genetically redundant roles [20,22,27]. Simultaneous reduction in BZR1 and BES1/BZR2 levels through RNAi led to a semi-dwarf phenotype [27]. Similarly, dominant mutations (*bes1-D* and *bzr1-D*) promoting a hypophosphorylated state of these two transcription factors, and hence to their constitutive activity, suppressed various phenotypic abnormalities in brassinosteroid-insensitive and deficient mutants and fully restored hypocotyl length in the dark [20,22,24]. When grown in light, *bes1-D* and *bzr1-D* mutants exhibit distinct morphological features, such as long and short leaf petiole length, respectively. Light-grown plants expressing the dominant form of BZR1-D under the BRI1 promoter, feature a phenotype similar to that of *bes1-D* [29]. Hence, the different phenotypic aspects of *bes1-D* and *bzr1-D* mutants could be a result of their distinct expression patterns, but this possibility has not been thoroughly addressed. Identifying their target genes and interacting partners has therefore been the subject of recent experiments, as discussed next.

3. Emerging brassinosteroid regulatory modules controlling cell elongation

Postembryonic growth involves consecutive stages of cell proliferation, elongation and subsequent differentiation. Although it is now established that brassinosteroid activity affects each of these processes, much of the brassinosteroid-related research has focused on cell elongation, as primarily inferred from changes in hypocotyl length, in whole-plant-based genomic studies (Fig. 1).

Recently, various labs performed global gene-expression profiling in response to exogenous application of brassinosteroids in the background of loss- and gain-of-function mutants. The various data sets revealed changes in the expression of hundreds of genes belonging to different functional groups, including prominent gene groups encoding for cell wall biosynthesis and remodeling enzymes as well as for cytoskeleton-related functions, consistent with the central role of brassinosteroids in cell elongation [30–34]. An additional group of genes linked brassinosteroids to hormonal and light signaling pathways, as exemplified by the negative regulation of many genes involved in chloroplast development and photomorphogenesis upon exposure to brassinosteroids [25,26]. These findings correlated with the ectopic expression of light-regulated genes previously observed in dark-grown brassinosteroid-deficient mutants [8]. Moreover, many brassinosteroid-responsive genes were shown to be co-regulated by either auxin or gibberellins. Indeed, physiological and genetic data revealed that brassinosteroid and auxin activities have interdependent and synergistic effects on gene expression and cell elongation, as will be elaborated further below [35–38]. In parallel, three studies have recently provided compelling evidence that gibberellin-driven control of cell elongation is brassinosteroid-dependent [39–41]. These works demonstrated that changes in gene expression and hypocotyl length triggered by gibberellins were abolished in brassinosteroid-deficient mutants, whereas gibberellin-deficient mutants largely responded to brassinosteroid treatment [39–41] (Fig. 1). A simple and elegant mechanism was proposed to explain this dependency, as will be briefly discussed below (Fig. 1).

In efforts to further understand the broad genomic impact of brassinosteroids and to delineate the direct primary effect of brassinosteroids, two genome-wide surveys were recently conducted to identify BZR1 and BES1/BZR2 targets [25,26]. These experiments were the first to establish the transcription factors directly regulated by BZR1 and BES1/BZR2 and to characterize the secondary effect of the latter on gene expression [25,26,42]. An intriguing molecular mechanism was described linking brassinosteroids, gibberellins, temperature and light-mediated signaling [39–42] (Fig. 1). In this framework, BZR1 forms heterodimers with the light- and temperature-regulated transcription factor PHYTOCHROME-INTERACTING FACTOR 4 (PIF4) and regulates the expression of genes necessary for cell elongation. This is largely achieved through their direct activation of secondary transcription factors belonging to the helix-loop-helix PACLOBUTRAZOLRESISTANCE (PRE) family [42]. The PRE family, via a series of posttranslational antagonistic interactions with other helix-loop-helix proteins (including ILI1 BINDING bHLH 1 (AtIBH1 [43]) and ATBS1 INTERACTING FACTORs (AIFs [44,45])), activates the binding of several basic-helix-loop-helix factors (bHLH) like HOMOLOG OF BEE2 INTERACTING WITH IBH1 (HBI1) and ACTIVATORS FOR CELL ELONGATION (ACEs), to promoters of cell wall component genes (Fig. 1) [45–47]. Under low gibberellin levels, DELLA, the negative regulator of the pathway, accumulates and binds BZR1 and PIF4, thereby inhibiting their activity and subsequently the induction of specific PREs [39–42,48] (Fig. 1). Hence, cell elongation is coordinated by various internal and environmental cues. The BZR1–PIF–DELLA module can account for cell elongation in hypocotyl and leaves. Whether it has the same effect in other organs, like roots, remains an open question.

As with BZR1, BES1/BZR2 binds PIF4 and DELLA (Fig. 1) but has also been shown to interact with other transcription regulators involved in cell elongation, including BES1-INTERACTING MYC-LIKE 1 (BIM1) [27], MYB30 [49], MYELOBLASTOSIS FAMILY TRANSCRIPTION FACTOR-LIKE 2 (MYBL2) [50] and histone-modifying enzymes [51,52]. It is unknown if BZR1 interacts with these factors.

Studies of crosstalk mechanisms between brassinosteroids and auxin revealed that gene expression in response to one hormone requires the activity of the other and that application of both hormones imparts a synergistic effect [35,36,38]. Attempts to address