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Brassinosteroids Regulate Root Growth, Development, and Symbiosis

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ABSTRACT

Brassinosteroids (BRs) are natural plant hormones critical for growth and development. BR deficient or signaling mutants show significantly shortened root phenotypes. However, for a long time, it was thought that these phenotypes were solely caused by reduced cell elongation in the mutant roots. Functions of BRs in regulating root development have been largely neglected. Nonetheless, recent detailed analyses, revealed that BRs are not only involved in root cell elongation but are also involved in many aspects of root development, such as maintenance of meristem size, root hair formation, lateral root initiation, gravitropic response, mycorrhiza formation, and nodulation in legume species. In this review, current findings on the functions of BRs in mediating root growth, development, and symbiosis are discussed.

Key words: brassinosteroids, root meristem, root hair, lateral root development, gravitropic response, symbiosis

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INTRODUCTION

Plant survival largely relies on an appropriate root system due to its mechanical supporting role and its function in water and nutrient acquisition. Plant root is also a major host for mycorrhizae and nitrogen-fixing microorganisms. The simple cellular organization of an *Arabidopsis* root makes it a preferred model for studying internal and external factors regulating root growth and development. In *Arabidopsis*, four radially symmetric layers, from outer to inner, including epidermis, cortex, endodermis, and pericycle, surround the vascular tissue in the middle of the root, composing a concentric cylinder primary root (Figure 1). The pericycle and the inner vascular tissues make up the stele.

An *Arabidopsis* root meristem contains a set of stem cells maintained by a quiescent center (QC), a group of three to four cells with very low proliferation activity. QC cells together with their surrounding stem cells are defined as stem cell niche, which provides new cells for a growing root. Initial cells shootward of the QC give rise to the stele. The cortex and endodermis initial cells located laterally outward to the QC give rise to the cells for the cortical and endodermal layers. The initial cells for the lateral root cap and epidermis located rootward and outward of the QC give rise to the lateral root cap and the epidermal layer. The columella stem cells (CSCs) on the rootward face of the QC produce the columella cells (Figure 1).

The *Arabidopsis* primary root is also patterned longitudinally along its apical-basal axis, including the root cap, meristematic zone, elongation zone, and differentiation zone (Figure 1). Stem cells in the meristematic zone at the root tip divide multiple times to generate a pool of cells that will elongate and differentiate. Shootward of the meristematic zone is the elongation zone where cells lose their ability to divide but increase in length by many times their width. In the differentiation zone, cells exhibit their mature characteristics and functions, for instance, the formation of root hairs from epidermal cells. The differentiation zone is also the site of emergence of lateral roots.

Growth and development of a plant root system needs coordinated regulation of endogenous cues as well as environmental signals. Previous studies demonstrated that plant root growth and development are inextricably linked with phytohormones (Pacifici et al., 2015). Brassinosteroids (BRs) are a class of polyhydroxylated steroidal hormones playing pivotal roles during many aspects of plant growth and development, such as cell elongation, cell division, senescence, vascular differentiation, reproduction, photomorphogenesis, and responses to various stresses (Clouse and Sasse, 1998; Divi and Krishna, 2009). A variety of BRs were identified in the roots of different plant species, such as maize and *Arabidopsis* (Yokota et al., 2001; Shimada et al., 2003; Kim et al., 2005b). Mutants impaired in BR biosynthesis or signal transduction

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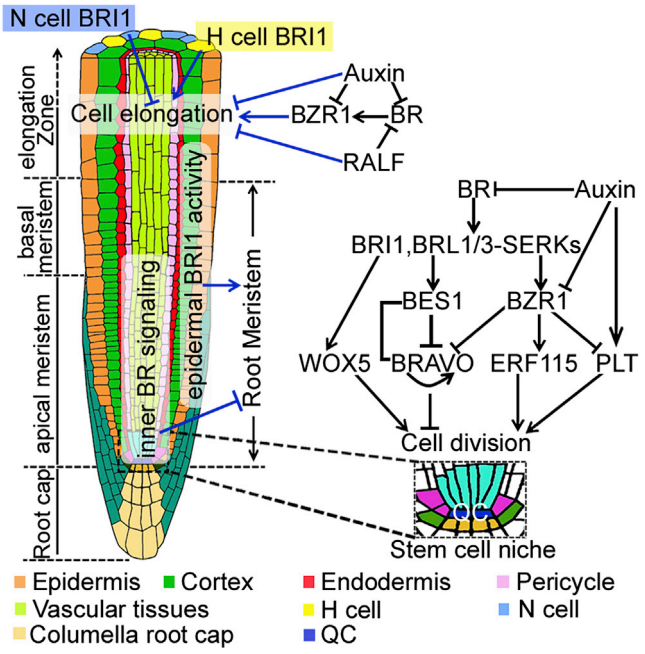

Figure 1. BRs Play Important Roles in Regulating Root Meristem Maintenance and Root Elongation.

The anatomy of an *Arabidopsis* root is shown. While BR signaling in the epidermis promotes stem cell proliferation to regulate root meristem size, BR signaling in the inner cells attenuates the effect of BRI1 in epidermal cells (left). In addition, BRs control root meristem size by directly regulating QC cell division (right). The functions of BRs in regulating root cell elongation are shown in the left panel. The expression of *BRI1* in hair cells or nonhair cells promotes or inhibits root elongation, respectively. BR and auxin antagonistically regulate the expression of BZR1-target genes to control root elongation. RALF antagonizes the action of BR in regulating root cell elongation. Arrows and bar ends indicate activation and inhibitory effects, respectively.

display a short-root phenotype (Li et al., 1996; Müssig et al., 2003). Physiological analyses indicated that supplementation of low concentrations of BRs can promote root growth, whereas application of high concentrations of BRs can inhibit root growth (Roddick et al., 1993; Clouse et al., 1996; Müssig et al., 2003). Recent studies suggested that BRs play important roles during root growth and development. Here we briefly summarize our current understanding of BR signal transduction and homeostasis, and discuss the roles of BRs and their interplays with other signaling pathways in regulating root growth and development.

CURRENT MODEL OF BR SIGNAL TRANSDUCTION

Mutants impaired in BR signal transduction, such as *bri1* and *bak1*, show a significantly shortened root phenotype (Clouse et al., 1996; Li et al., 2002). Interestingly, it was reported that some gain-of-function BR mutants, such as *bes1-D* or *BRI1* overexpressors, also produce roots shorter than those of wild-type plants (González-García et al., 2011). These observations suggest that an appropriate intensity of BR signaling is important for optimal plant root growth and development.

The BR signaling pathway has been well established through combinational approaches from genetics, biochemistry, and "omics." BRs are perceived by three leucine-rich repeat receptor-like kinases (LRR-RLKs) including the main receptor BRASSINOSTEROID-INSENSITIVE 1 (BRI1) and its two paralogs, BRI1-LIKE 1 (BRL1) and BRL3 (Clouse et al., 1996; Li and Chory, 1997; He et al., 2000; Wang et al., 2001; Caño-Delgado et al., 2004; Zhou et al., 2004; Kinoshita et al., 2005). It was found that BR binding to the extracellular domain of BRI1 activates its cytoplasmic kinase domain, which phosphorylates a downstream negative regulator BRI1 KINASE INHIBITOR 1 (BKI1) (Wang and Chory, 2006). The phosphorylated BKI1 dissociates from the plasma membrane, allowing BRI1 to recruit its co-receptor BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1), an LRR-RLK, distinctive from BRI1 (Li et al., 2002; Nam and Li, 2002; Wang and Chory, 2006; Jaillais et al., 2011). BAK1 belongs to the SOMATIC EMBRYOGENESIS RECEPTOR KINASES (SERKs) subfamily, which contains five members in *Arabidopsis*, and BAK1 is also designated as SERK3 (Hecht et al., 2001; Li et al., 2002; Nam and Li, 2002; Gou et al., 2012). Genetic studies demonstrated that SERKs are indispensable to the early events of the BR signaling pathway (Gou et al., 2012). Brassinolide (BL) is the final product of the BR biosynthesis pathway and the most active form of BRs. Recent crystallographic studies demonstrated that the extracellular domains of BAK1 and SERK1 are directly involved in BL binding during ligand and receptor recognition, which are consistent with the genetic results (Gou et al., 2012; Santiago et al., 2013; Sun et al., 2013). BAK1 or SERK1 alone, however, does not show BL binding activity. Interaction of BL with its receptor BRI1 generates a new surface, which allows the interaction of the receptor-ligand complex with its co-receptor BAK1. Sequential transphosphorylation between BRI1 and BAK1 activates BRI1 completely (Wang et al., 2008). The BRI1-BL-BAK1 complex then can initiate a downstream signaling cascade.

The activated BRI1 phosphorylates membrane-bound receptor-like cytoplasmic kinases (RLCKs), such as BR-SIGNALING KINASE 1 (BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH 1 (CDG1) (Tang et al., 2008; Kim et al., 2011). These RLCKs then activate BRI1-SUPPRESSOR 1 (BSU1), a PP1-type phosphatase possessing tyrosine phosphatase activity, which dephosphorylates a phosphotyrosine residue (at position 200) of a key negative regulator, BRASSINOSTEROID-INSENSITIVE 2 (BIN2), leading to inactivation of BIN2 (Li and Nam, 2002; Mora-García et al., 2004; Kim et al., 2009, 2011). BIN2 is a GSK3-like kinase and its kinase activity can be inhibited by bikinin, a specific GSK3 kinase inhibitor (Li et al., 2001; De Rybel et al., 2009). These reversible phosphorylation and dephosphorylation events result in nuclear accumulation of two unphosphorylated transcription factors, BRASSINAZOLE-RESISTANT 1 (BZR1) and BRI1-EMS-SUPPRESSOR 1 (BES1, also named BZR2) (He et al., 2002; Wang et al., 2002; Yin et al., 2002). BZR1 and BES1 can activate or repress the expression of hundreds of their target genes in the genome to mediate many aspects of plant growth and development (Sun et al., 2010; Yu et al., 2011).

CONTROL OF BR HOMEOSTASIS

Mutants that are defective in BR biosynthesis, such as *det2* and *cpd*, show much shorter roots than those of wild-type plants,

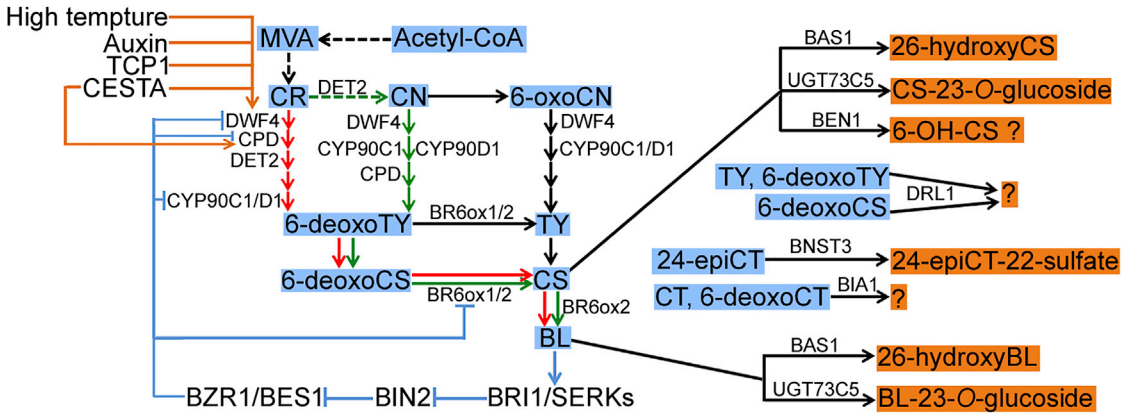

Figure 2. BR Homeostasis Is Dynamically Regulated.

Both BR biosynthesis and catabolism are critical for BR homeostasis. A simplified BR biosynthesis pathway is shown from acetyl-CoA to BL. The BR biosynthetic intermediates and final product are shown in blue boxes. The latest known eight-step predominant pathway for BL synthesize is indicated by red arrows. The CN-dependent pathway is indicated by green arrows. The biosynthetic enzymes are marked alongside the arrows. A simplified BR signaling pathway is also shown. Blue arrows and bar ends indicate activation and inhibitory effects, respectively. Orange arrows represent positive transcriptional regulation of BR biosynthetic genes. BR metabolism and the enzymes involved are shown in the right panel. The inactivated products are shown in orange boxes. The question marks represent hypothetical reactions which have not been confirmed in plants.

which can be rescued by exogenous application of BRs (Chory et al., 1991; Fujioka et al., 1997). In addition, BRs control plant root growth and development in a concentration-dependent manner (Roddick et al., 1993; Clouse et al., 1996; Müssig et al., 2003). Homeostasis therefore is critical for appropriate functions of BRs during plant root growth and development.

BR biosynthesis and inactivation are thought to directly affect BR homeostasis (Figure 2). Analytic chemistry using suspension cultures and genetic analyses using *Arabidopsis* mutants contributed greatly to elucidate the entire BR biosynthetic pathway (Noguchi et al., 2000; Zhao and Li, 2012). It is believed that BRs are synthesized via a secondary metabolic pathway. Acetyl-CoA is converted to mevalonate (MVA) through many different reactions. MVA is then converted to campesterol (CR) via multiple steps. CR is thought to be the first molecule specifically entering the BR biosynthetic pathway. Plants have evolved several ways to synthesize BL from CR. CR is first converted to campestanol (CN), then to castasterone (CS) through an early and a late C-6 oxidation pathway (Suzuki et al., 1994a, 1994b; Fujioka et al., 1995; Choi et al., 1996, 1997). Both the early and the late C-6 oxidation pathways were found throughout the plant kingdom, while the latter is more prevalent. CS is ultimately converted to BL (Yokota et al., 1990; Suzuki et al., 1993). This route is also known as a CN-dependent pathway (Ohnishi et al., 2012). Recent analyses identified a CN-independent pathway, through which CR is converted to 6-deoxotyphasterol (6-deoxoTY), an intermediate of the late C-6 oxidation pathway, then to BL through the late C-6 oxidation pathway (Fujioka et al., 2002; Ohnishi et al., 2012). This route consists of eight steps and is thought to be the predominant BR biosynthetic pathway (Ohnishi et al., 2012). Several genes encoding key BR biosynthetic enzymes have also been cloned. For example, *DE-ETIOLATED 2* (*DET2*) encodes a 5α-reductase and was found to be involved in early 5α-reduction steps during BR biosynthesis (Li et al., 1996; Fujioka et al., 1997; Noguchi et al., 1999; Fujioka et al., 2002). There are several cytochrome P450 enzymes playing important roles in BR biosynthesis, including CONSTITUTIVE PHOTOMORPHOGENESIS AND DWARFISM (CPD), DWARF4 (DWF4), ROTUNDIFOLIA3 (ROT3, also named CYP90C1), CYP90D1, BRASSINOSTEROID-6-OXIDASE 1 (BR6ox1), and BR6ox2. Recent data proved that CPD catalyzes a C-3 oxidation of the early BR biosynthetic intermediates, which is different from a previous report suggesting that CPD participates in a C-23 hydroxylation step in BR biosynthesis (Szekeres et al., 1996; Ohnishi et al., 2012). Genetic and biochemical analyses indicated that ROT3 and its homolog CYP90D1 redundantly catalyze the C-23 hydroxylation steps (Ohnishi et al., 2006). DWF4, a C-22 hydroxylase, is responsible for multiple C-22 hydroxylation steps (Choe et al., 1998), which are considered to be the rate-limiting steps in the BR biosynthetic pathway (Kim et al., 2006b). Both BR6ox1 and BR6ox2 catalyze multiple C-6 oxidation reactions. BR6ox2, instead of BR6ox1, is involved in converting CS to BL (Bishop et al., 1999; Shimada et al., 2001; Kim et al., 2005a). Manmade chemicals that can directly inhibit BR biosynthesis have also been found. For example, brassinazole (BRZ), a triazole-type chemical, was found to target DWF4 to inhibit BR biosynthesis (Asami et al., 2000, 2001).

Excess or lack of BRs is detrimental to plant growth and development. Plants therefore must monitor and tightly regulate BR biosynthesis and catabolism. Consequently, plants evolved a feedback loop to control the rate of BR biosynthesis. Expression levels of several BR biosynthetic genes were found to be downregulated by exogenous BL application (Mathur et al., 1998; Bancos et al., 2002). BES1 and BZR1 are directly involved in repressing the expression of *DWF4*, *CPD*, and other biosynthesis genes when endogenous BRs have reached a level high enough to maintain normal growth and development (He et al., 2005; Sun et al., 2010; Yu et al., 2011). When additional BRs are needed at certain developmental stages or under certain conditions, plants need appropriate mechanisms to quickly trigger the production of BRs. Accelerating the biosynthetic rate is thought to be an effective way to accumulate BRs. Genetic analyses also identified a number of

factors positively mediating BR biosynthesis. For instance, TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1 (TCP1) and CESTA, two basic helix-loop-helix (bHLH) transcription factors, were identified as positive regulators of BR biosynthetic genes. TCP1 upregulates the expression of *DWF4*, whereas CESTA elevates the expression of *CPD* by directly binding to GGNCCC and G-box motifs in their promoter regions, respectively (Guo et al., 2010; Poppenberger et al., 2011; Gao et al., 2015). It was found that auxin or high temperature can also induce *DWF4* expression (Chung et al., 2011; Maharjan and Choe, 2011).

In addition, BR catabolism plays a critical role in maintaining the homeostasis of endogenous bioactive BRs. Plants developed many strategies to inactivate bioactive BRs. For example, PHYB ACTIVATION-TAGGED SUPPRESSOR1 (BAS1), a cytochrome P450, acts as a C-26 hydroxylase converting both CS and BL to their C-26 hydroxylated derivatives (Neff et al., 1999). BRASSICA NAPUS SULFOTRANSFERASE 3 (BNST3), a steroid sulfotransferase identified in *Brassica napus*, catalyzes the O-sulfonation of BRs at the 22-OH position (Rouleau et al., 1999). A UDP-glycosyltransferase named UGT73C5 catalyzes the 23-O-glucosylation of CS and BL (Poppenberger et al., 2005). DWARF AND ROUND LEAF-1 (DRL1), a putative CoA-dependent acyltransferase, is involved in BR metabolism likely by promoting esterification of certain BRs in *Arabidopsis* (Zhu et al., 2013). *bri1-5* ENHANCED1 (BEN1) and BRASSINOSTEROID INACTIVATOR1 (BIA1) may also be involved in inactivating BRs via mechanisms yet to be elucidated (Yuan et al., 2007; Roh et al., 2012).

BRs REGULATE ROOT MERISTEM SIZE

Precise regulation is required to ensure specification of the stem cell niche and maintenance of the undifferentiated state of the initial cells. Several transcription factors are found to be important regulators for the identity of root stem cells. PLETHORA (PLT) transcription factors, members of the AP2 transcription factor family, are essential for the maintenance of root stem cell niche (Aida et al., 2004; Galinha et al., 2007). The expression of *PLT*s can be induced by auxin and their expression domains are defined by local auxin accumulation (Aida et al., 2004; Blilou et al., 2005; Mähönen et al., 2014). A parallel mechanism for root stem cell maintenance involves two GRAS transcription factors, SHORT ROOT (SHR) and SCARECROW (SCR) (Di Laurenzio et al., 1996; Helariutta et al., 2000; Sabatini et al., 2003; Levesque et al., 2006; Cui et al., 2007). *SHR* is expressed in stele and its encoded protein moves to the adjacent cells, including QC, to activate the expression of *SCR* (Helariutta et al., 2000; Levesque et al., 2006; Cui et al., 2007). *SCR* expression in QC maintains the identity of QC and stem cells (Sabatini et al., 2003). Another component required in the maintenance of stem cell identity is WUSCHEL-RELATED HOMEOBOX 5 (WOX5), a homeodomain transcription factor, whose expression can be restricted in QC by the CLAVATA3/EMBRYO SURROUNDING REGION 40 (CLE40)-ARABIDOPSIS CRINKLY 4 (ACR4) signaling pathway (Haecker et al., 2004; Sarkar et al., 2007; Stahl et al., 2009). Auxin can also restrict the expression of *WOX5* in QC via AUXIN RESPONSE FACTOR 10 (ARF10) and ARF16. However, WOX5, ARF10, and ARF 16 each provide independent inputs to restrict CSC fate (Ding and Friml, 2010; Bennett et al., 2014). Recent studies found that WOX5 can move from the QC to the CSCs to maintain the undifferentiated state of these cells (Pi et al., 2015).

Root length is determined by the number of cells and their final length. BRs affect both cell proliferation and cell elongation in a concentration-dependent manner to control root meristem size. For example, mutants impaired in BR signaling, such as *bri1-116*, displayed a short-root phenotype due to abnormal cell cycle progression and reduced cell expansion rate (González-García et al., 2011; Hacham et al., 2011). A very low concentration of BL promotes root growth, whereas a high concentration of BL ($\geq$0.04 nM) inhibits root growth through the control of root meristem size (González-García et al., 2011). Consistently, cell organization and cell length in the short roots of *dwf4*, a BR-deficient mutant, can be rescued by an exogenously applied low concentration of BL, but not a high concentration of BL, even for a relative long period of time (Chaiwanon and Wang, 2015).

BRs regulate root meristem size maintenance depending on their action site. For instance, targeted *BRI1* expression in epidermis can completely rescue the meristem size of *bri1-116*, whereas *BRI1* expression in the inner cell files failed to significantly alter the meristem size of *bri1-116*, indicating that BR perception in the epidermis is sufficient to control root meristem size (Hacham et al., 2011). Expression of *AGL42*, a BL-inducible QC marker, was dramatically reduced in *bri1* mutant and could be restored by epidermal *BRI1* expression, but not by direct BRI1 activity in QC and stele or by exogenous BL treatment, indicating that epidermal cell-expressed *BRI1* controls gene expression in inner cell files (Hacham et al., 2011). Consistently, expression of *bzr1-1D* (an activated and hypophosphorylated form of *BZR1*) in the epidermis of *bri1-116* promoted root meristem growth, whereas expression of *bzr1-1D* in endodermis or QC showed no effect on the meristem size of *bri1-116* (Wang et al., 2002; Chaiwanon and Wang, 2015). In summary, the aforementioned results demonstrate that epidermal BR signaling is sufficient to maintain root meristem development (Figure 1).

The spatial distribution of BR signaling can trigger opposing impacts on root meristem size. Epidermal *BRI1* expression in the *bri1* background caused slightly enlarged meristem compared to that of wild-type (Hacham et al., 2011). Interestingly, this enlarged meristem phenotype was enhanced in a *bri1 brl1 brl3* triple mutant (Vragovic et al., 2015), suggesting a buffered role of vascular BR signaling, mediated by BRL1 and BRL3, on the epidermal BR effect on meristem size. In addition, BRI1 activity in epidermis promotes cell proliferation, but BRI1 activity in stele promotes cell differentiation. Furthermore, a tissue-specific translatome profiling assay found that BR-induced genes were mainly expressed in the basal meristem of epidermis, however, BR repressed genes were enriched in the apical meristem of stele (Vragovic et al., 2015). Consistently, BZR1-YFP is accumulated at a low level in the nuclei of stem cells but at a high level in the nuclei of epidermal cells in the transition and elongation zones (Chaiwanon and Wang, 2015). Taken together, these results indicate that BR signaling in different cell files has a contrasting effect on the root meristem size (Figure 1).

QC is essential for the specification of stem cell niche and the maintenance of the undifferentiated state of stem cell initials. BRs are required to control QC identity and stem cell activity and thus root meristem size (Figure 1). For instance, the expression of *WOX5* was low in *bri1-116* roots, while the number of cells expressing *WOX5* was increased in *bes1-D* and *BRI1*-overexpressing plants (González-García et al., 2011). Consistently, BL treatment can increase the expression domain of *WOX5* (González-García et al., 2011), whereas BRZ treatment can reduce the expression level of *WOX5* in stem cell niche (Hacham et al., 2011). Genetic and biochemical analyses revealed that BRL1, BRL3, and BAK1 interact with one another to mediate QC organization (Fàbregas et al., 2013). Different from BRI1, BRL1 and BRL3 are confined in stem cell niche and stele at a high level, which can be broadly detected in root meristem, but reduced in QC (Fàbregas et al., 2013). Whereas a reduction in the frequency of QC division was observed in *brl1 brl3* double mutant roots, they were of similar length to wild-type ones. In addition, the roots of *bri1-301 brl1 brl3* and *bri1-116 brl1 brl3* triple mutants were of a similar length to those of their respective *bri1* parents, suggesting BRL1 and BRL3 contribute differently from BRI1 to root growth.

Some downstream targets of BR signaling in regulating QC activity have been uncovered (Figure 1). For example, BRASSINOSTEROIDS AT VASCULAR AND ORGANIZING CENTER (BRAVO), also known as MYB56, is an R2R3-MYB transcription factor specifically expressed in the vascular initials and QC cells. *bravo* mutants show strong dividing QCs, whereas inducible overexpression of *BRAVO* represses root growth (Vilarrasa-Blasi et al., 2014). *BRAVO* can be transcriptionally regulated by itself (Vilarrasa-Blasi et al., 2014). BRs mediate QC identity through BES1, which counteracts the action of BRAVO by not only downregulating its transcript level but also heterodimerizing with it (Vilarrasa-Blasi et al., 2014). BRs also induce expression of *ETHYLENE RESPONSE FACTOR 115* (*ERF115*), which upregulates the expression of *PSK5*, a gene encoding a peptide hormone, to enhance QC cell divisions (Yang et al., 2001; Kutschmar et al., 2009; Heyman et al., 2013). BZR1-mediated BR signaling also promotes QC cell division by downregulating expression of *BRAVO* and upregulating *ERF115* (Lee et al., 2015). Transcriptome analysis found that the expression of *PLTs* is autonomously regulated by the local BR signaling in the stele (Vragovic et al., 2015). Consistently, ectopic expression of *bzr1-1D* in QC increased division of QC cells in a cell-autonomous manner (Chaiwanon and Wang, 2015). In addition, the BZR1-target genes expressed in QC and surrounding stem cells, including *PLTs* and *BRAVO*, are mostly repressed by BR but induced by auxin, indicating an antagonistic effect of BR and auxin on QC cell division (Chaiwanon and Wang, 2015).

BRs also play a critical role in regulating the maintenance and differentiation of distal CSCs in a concentration- and BZR1-/BES1-dependent manner. BRs inhibit stem cell differentiation at low concentrations and promote it at higher concentrations (González-García et al., 2011; Lee et al., 2015). Previous observation of mPS-PI-stained root tips of Col-0 and *bes1-D* indicated that BES1-mediated BR signaling promotes differentiation of the distal CSCs (González-García et al., 2011), whereas BZR1-mediated BR signaling, in contrast to BES1-mediated signaling, inhibits the differentiation of distal meristem, thus delaying the development of starch-filled columella cells (Lee et al., 2015).

Taken together, these studies showed that BRs affect root meristem size via different mechanisms (Figure 1). First, BRs promote cell expansion and maintain normal cell numbers in the root meristem. Second, BRs are important to maintain QC identity. Third, BRs promote and inhibit differentiation of distal CSCs via BES1- and BZR1-mediated signaling, respectively. In addition, the promoting and inhibiting effects of BRs on root meristem size largely depend on hormonal concentration and their action location.

BRs MEDIATE ROOT CELL ELONGATION

Cell elongation contributes significantly to the final length of a root. The extensibility of cell wall is an important determinant in regulating cell elongation. Cellulose is the major load-bearing component of the cell wall. Reduction of crystalline cellulose to amorphous cellulose promotes unidirectional cell expansion, whereas accumulation of crystalline cellulose limits unidirectional cell expansion (Fujita et al., 2011; Fridman et al., 2014).

BRs show cell-type-specific functions on cell elongation (Figure 1) (Fridman et al., 2014). Ectopic expression of *BRI1* in hair cells promotes the elongation of all different cell types within the root elongation zone, whereas expression of *BRI1* in nonhair cells inhibits root cell elongation, suggesting that the spatial distribution rather than an absolute level of BRI1 determines root cell elongation (Fridman et al., 2014). In addition, BRI1 activity in nonhair cells upregulates the expression of ethylene biosynthetic genes, resulting in an increase of ethylene and subsequent accumulation of crystalline cellulose in the cell wall of nonhair cells, which impairs unidirectional cell elongation and therefore inhibits overall root elongation (Fridman et al., 2014). Consistently, targeted expression of *bzr1-1D* in epidermis can rescue the cell length within the elongation zone, and partially rescue the final root length of *bri1-116*. However, expression of *bzr1-1D* in endodermis and QC had no effect on cell elongation or root length (Chaiwanon and Wang, 2015).

BRs and auxin counteract each other via BZR1 during root cell elongation (Figure 1). BZR1 accumulates at a higher level in the nuclei of epidermal cells in the transition and elongation zones to activate most of its target genes, which are involved in cell wall organization and biogenesis, suggesting their roles in regulating cell elongation. Genetic and physiological assays demonstrate that BR and auxin show opposite effects on the expression of these genes to antagonistically control root elongation (Chaiwanon and Wang, 2015). However, a previous report found that the epidermal BRI1 activity elevates auxin levels to promote cell proliferation in the meristem zone (Vragovic et al., 2015). The mechanisms balancing the interactions of BRs and auxin in different tissues need to be elucidated in the future.

RAPID ALKALINIZATION FACTOR (RALF), a peptide hormone, suppresses cell elongation of primary roots by activating a cell surface receptor FERONIA in *Arabidopsis* (Pearce et al., 2001; Covey et al., 2010; Mingossi et al., 2010; Haruta et al., 2014).

Overexpression of *AtRALF1* resulted in a phenotype with reduced root cell size, while silencing *AtRALF1* promoted root elongation via increasing the size of root cells (Bergonci et al., 2014). Transgenic plants overexpressing *AtRALF1* showed reduced sensitivity to BL. Simultaneous treatment of RALF and BL resulted in reduced expression levels of the RALF-inducible genes related to cell wall rearrangement and BR biosynthesis (Bergonci et al., 2014). These results support that RALF antagonizes the action of BR in *Arabidopsis* roots, and the interplay of these two hormones determines final cell expansion (Figure 1).

BRs ARE INVOLVED IN ROOT HAIR FORMATION

A root hair is a thin tubular structure that forms from an epidermal hair cell in the differentiation zone. The formation of root hairs can greatly increase the surface area of a root, resulting in dramatically elevated uptake of water and nutrients from the surrounding environment. The *Arabidopsis* root epidermal cells in the differentiation zone differentiate into hair cells or nonhair cells based on their positions relative to the underlying cortical cells (Ishida et al., 2008; Grebe, 2012). Generally, an epidermal cell located outside the cleft separating two cortex cells (H position) develops to a hair cell (H cell), whereas an epidermal cell adjacent to a single cortex cell (N position) often becomes a nonhair cell (N cell) (Ishida et al., 2008; Grebe, 2012). Numerous genetic and molecular studies have generated a fairly clear diagram of the genes responsible for root cell fate determination. In N cells, a transcription factor complex, consisting of WEREWOLF (WER), GLABRA3 (GL3), ENHANCER OF GLABRA3 (EGL3), and TRANSPARENT TESTA GLABRA 1 (TTG1), upregulates the expression of *GLABRA2* (*GL2*) and *CAPRICE* (*CPC*), two genes encoding for a homeodomain transcription factor and a single-repeat MYB protein, respectively (Galway et al., 1994; Wada et al., 1997; Lee and Schiefelbein, 1999; Bernhardt et al., 2003; Zhang et al., 2003; Ryu et al., 2005; Shen et al., 2006; Song et al., 2011). WER is an R2R3 MYB-domain protein (Lee and Schiefelbein, 1999). GL3 and its homolog EGL3 belong to the family of bHLH transcription factors, which are transcribed in H cells and moved into their adjacent N cell nuclei to determine N cell fate (Bernhardt et al., 2003, 2005; Cheng et al., 2014). TTG1 is a WD-40 repeat transcription factor (Galway et al., 1994). Activation of *GL2* expression by this WER-GL3/EGL3-TTG1 module leads to N cell fate (Shen et al., 2006; Schiefelbein et al., 2009). CPC can travel laterally into the neighboring presumptive H cells where it competes with WER for its binding with the GL3/EGL3-TTG1 complex, which is unable to induce *GL2* expression (Wada et al., 1997; Ryu et al., 2005; Song et al., 2011). SCRAMBLED (SCM) is an LRR-RLK specifically activated in H cells by an unknown signal from the junction of two overlying cortical cells (Kwak et al., 2005). It was predicted that the activated SCM promotes H cell fate by reducing the abundance of WER (Kwak et al., 2005).

BRs are essential for position-dependent epidermal cell fate specification in roots. Quantitative RT-PCR analyses indicated that exogenous BL treatment can induce the expression of *WER* and *GL2*, while the BR-insensitive mutant *bri1* displayed a reduced expression level of *WER* and *GL2* (Kuppusamy et al., 2009). Transverse sections showed that *GL2* was aberrantly expressed in some H-position cells of the *bri1* roots but was not lost in N cells. Consistently, more cells in the H positions developed into N cells when the BR signaling pathway was blocked, whereas blocking BR signaling had only a modest effect on the number of H cells formed in the N positions (Kuppusamy et al., 2009).

Data from a more recent publication, however, showed that BR signaling has an important role in suppressing H-cell fate and promoting N-cell fate in both the N and the H positions (Cheng et al., 2014). It was found that the relative hair numbers are higher in BR-deficient mutants but lower in BR signaling-enhanced plants compared with wild-type. Plants treated with BL or bikinin produced fewer root hairs in the H positions, while BRZ treatment caused more root hairs in the N positions (Cheng et al., 2014). Expression pattern analyses of *pGL2:GUS* showed that more N-cell-fate cells in *bri1-116* and *det2-1* roots lacked *GL2* expression (Cheng et al., 2014). However, more H-position cells in the roots of BR signaling-enhanced mutants ectopically expressed *GL2* (Cheng et al., 2014). Biochemical studies found that BIN2 can phosphorylate EGL3 and TTG1. The phosphorylation of EGL3 may help its movement from H cells to N cells. The phosphorylated TTG1 attenuate the transcriptional activity of the WER-GL3/EGL3-TTG1 complex (Cheng et al., 2014).

Taken together, in the presence of BRs, activated *WER* expression and inhibited BIN2 kinase activity facilitate formation of WER-GL3-TTG1 and WER-EGL3-TTG1 complexes in H cells and N cells, respectively. The WER-GL3-TTG1 and WER-EGL3-TTG1 complexes are activated to promote *GL2* expression and N-cell fate. Meanwhile, the BR signaling in N-position cells results in accumulation of CPC. CPC will then move into neighboring H-position cells to not only inhibit the expression of *WER* and *GL2* but also promote SCM accumulation. SCM further represses WER activity, reinforcing inhibition of *GL2* expression in H-position cells. Yet, the local BR signaling in H-position cells enhances the activity of WER-GL3-TTG1, which will promote *GL2* expression (Figure 3). Detailed molecular mechanisms of BRs in regulating root hair formation need to be elucidated in the near future.

BRs REGULATE LATERAL ROOT INITIATION

Lateral roots are critical in determining root system architecture. Lateral roots not only facilitate the efficiency of water uptake and acquisition of nutrition from the surrounding soil but also provide enough mechanical support for the aerial part of a plant (Lynch, 1995). Lateral roots are formed throughout the entire life span of a plant, which is different from the formation of a primary root that originates during embryogenesis. In *Arabidopsis*, a lateral root initiates from a pericycle founder cell located opposite the xylem poles (Dolan et al., 1993). The pericycle founder cell undergoes several rounds of oriented cell division and expansion to form a lateral root primordium, which then emerges from the parental root by cell expansion. After it has emerged, the lateral root meristem is activated and begins to

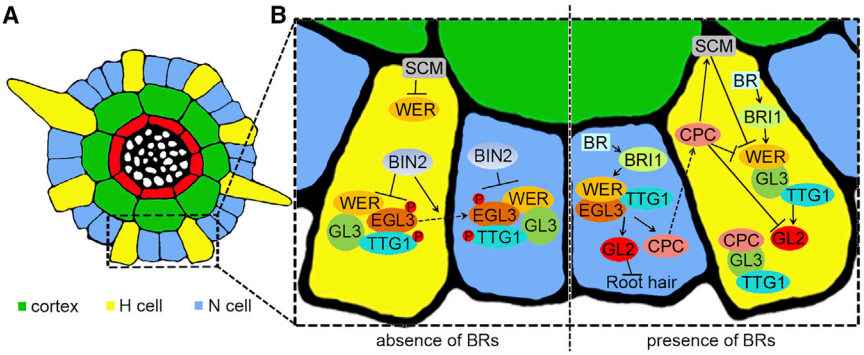

Figure 3. BRs Regulate Epidermal Cell Fate in Root.

(A) Schematic diagram showing transverse structures of the differentiation zone of an *Arabidopsis* root.

(B) A current model showing how BRs regulate root epidermal cell fate. In the absence of BRs, BIN2 phosphorylates EGL3, facilitating its movement from H cells to N cells. BIN2 phosphorylating TTG1 attenuates the activity of the WER-GL3/EGL3-TTG1 complex. In the presence of BRs, *WER* expression is activated and BIN2 kinase activity is inhibited. Thus, the WER-GL3/EGL3-TTG1 complexes are activated to promote *GL2* expression in N-position cells. BR signaling in N-position cells results in accumulation of CPC, which then moves into neighboring H-position cells to inhibit the expression of *WER* and *GL2* and promote SCM accumulation. SCM further represses WER activity, reinforcing the inhibition of *GL2* expression in H-position cells. Arrows and bar ends indicate activation and inhibitory effects, respectively.

direct the growth of the lateral root (Péret et al., 2009). Auxin plays a major role during lateral root development (Lavenus et al., 2013). Auxin gradient, with the maximum level at the root tip, is important for lateral root initiation and primordium development (Benková et al., 2003). The IAA28-ARF5, 6, 7, 8, 19 auxin signaling modules were found to be involved in the specification of lateral root founder cell identity (De Rybel et al., 2010). Auxin in the founder cells activates both SLR/IAA14-ARF7, 19, and BDL/IAA12-MP/ARF5 modules to control proper lateral root initiation and organization (Fukaki et al., 2002; Okushima et al., 2005; De Smet et al., 2010). The SHY2/IAA3-ARF7 module not only inhibits lateral root initiation by affecting auxin homeostasis but also regulates the development and emergence of lateral root primordium (Goh et al., 2012).

BRs regulate lateral root development through interacting with auxin (Figure 4). The BR-insensitive mutant *bri1* displayed a decreased number of lateral roots. Seedlings grown in MS medium containing low concentrations of BL (1–100 nM) exhibited an increased number of lateral roots (Bao et al., 2004). *DR5::GUS* is expressed at all stages of lateral root development and thus facilitates the identification of early lateral root primordia (Benková et al., 2003). The observation of the *DR5::GUS* expression pattern of transgenic seedlings treated with 50 nM BL showed that BL promoted the initiation of lateral root primordia but did not affect later lateral root development (Bao et al., 2004). Low concentrations of BL promote lateral root initiation by increasing acropetal auxin transport. At a low level of auxin, BL application promotes the effect of auxin on lateral root formation. However, this effect can be inhibited by imposition of NPA, an auxin transport inhibitor. An auxin transport assay showed that BRs indeed affect auxin acropetal transport (Bao et al., 2004). Higher concentrations of BL (100 nM and 1 μM) suppress lateral root formation (Gupta et al., 2015). Aux/IAAs act as repressors of auxin-responsive gene expression. A high concentration of BL (1 μM) can significantly induce expression of several Aux/IAA genes, such as AXR2/IAA7, SLR/IAA14, IAA28, and AXR3/IAA17, which were found to be involved in different development stages of lateral root formation (Kim et al., 2006a). These results indicated that BRs (at high concentrations) inhibit lateral root formation possibly via inducing the expression of *IAAs* to inhibit auxin signaling (Figure 4). More studies are needed to elucidate the mechanism mediating interactions between BR and auxin during lateral root development.

BRs also interplay with other signals to coordinately control lateral root development (Figure 4). Glucose promotes lateral root emergence at low concentrations but inhibits this process at high concentrations (Mishra et al., 2009; Gupta et al., 2015). Genetic and physiological studies found that BRs act downstream of glucose at low concentrations by increasing auxin transport and thus promote lateral root emergence (Gupta et al., 2015). RALF was proven to be involved in regulating lateral root formation as well as root elongation. Silencing the *AtRALF1* gene in *Arabidopsis* increased the lateral root number, while *AtRALF1* overexpression was shown to have the opposite effect. BL treatment had no effect on the number of lateral roots of *AtRALF1* overexpressors (Bergonci et al., 2014). These results indicated an opposing effect of AtRALF1 and BL during lateral root formation. Cytokinin inhibits the formation and the growth of lateral roots by disturbing cell division activity and the auxin gradient in pericycle founder cells (Li et al., 2006; Laplaze et al., 2007). Mutation of the cytokinin receptors AHK2 and AHK3 caused a high density of lateral roots. An *ahk2 ahk3* double mutant showed increased sensitivity to BL on lateral root growth (Chang et al., 2013). These results provided strong evidence that cytokinin antagonizes the BR effect on lateral root development through AHK2 and AHK3.

In conclusion, BRs play vital roles in regulating the initiation of lateral root primordia rather than affecting the later developmental stages of lateral roots. BR concentration determines either promotion or inhibition of lateral root initiation. BRs interact with other signals, such as auxin, glucose, peptide, and cytokinin, to coordinately control lateral root development (Figure 4).

BRs REGULATE ROOT RESPONSES TO GRAVITY

Root development is highly responsive to fluctuated environmental conditions including gravity, drought, temperature, and nutrients. Gravity is perceived by columella cells in root cap.

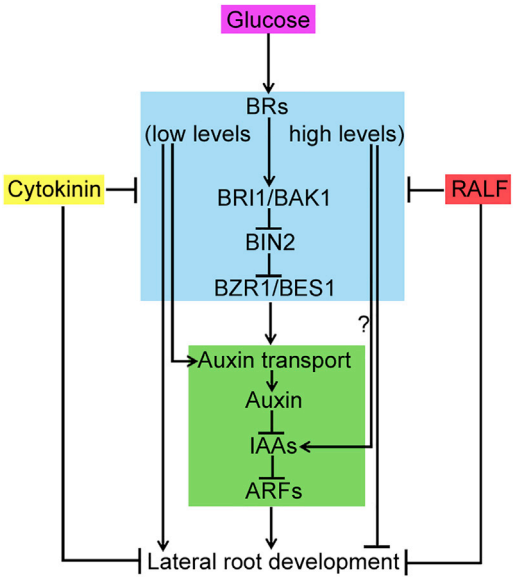

Figure 4. BRs Interplay with Other Signals during Lateral Root Development.

BRs interact with auxin during lateral root development. Low levels of BRs promote the effect of auxin on lateral root development via regulating auxin transport. A high concentration of BRs inhibits lateral root formation possibly via inducing the expression of *IAAs*, negative regulators of auxin signaling. Glucose acts through BRs to increase auxin transport to regulate lateral root development. Cytokinin and RALF act antagonistically with BRs to inhibit lateral root formation. Arrows and bar ends indicate activation and inhibitory effects, respectively.

Roots modulate their growth toward gravity, relying on auxin gradients to secure uptake of water and nutrients as well as anchorage in the soil (Vieten et al., 2007; Sato et al., 2015).

Exogenous BL application could increase the gravitropic curvature of primary roots in different species, such as maize and *Arabidopsis* (Kim et al., 2000, 2007; Li et al., 2005). In *Arabidopsis*, BR signaling mutants exhibited a reduced gravitropic curvature, whereas transgenic plants overexpressing *BRI1* displayed a greater gravitropic curvature than wild-type plants (Kim et al., 2007). BR functionally associates with auxin in regulating plant root gravitropism. First, low levels of IAA increase the effect of BL on root gravitropic response and vice versa (Kim et al., 2000, 2007; Li et al., 2005). However, higher concentrations of IAA can attenuate the effect of BL on root gravitropism (Kim et al., 2007). Second, exogenously applied BL can increase root gravitropic response via promoting ROP2 GTPase activity, which enhances the polar accumulation of PIN2 in root meristem and alters the distribution of auxin in plant roots (Li et al., 2005). Third, genetic studies showed that BR can induce actin cytoskeleton configuration in a way similar to that of auxin via altering PIN2 polar localization and auxin gradients to modulate root gravitropic responses (Lanza et al., 2012).

Glucose can influence root directional response via modulating BR signaling (Singh et al., 2014). The presence of BRs along with glucose synergistically induced root growth deviation from the vertical position. Roots of *bzr1-1D* displayed a magnified glucose-mediated root growth deviation, whereas inhibition of BR biosynthesis using BRZ could strongly reduce glucose-mediated root growth deviation (Singh et al., 2014). It was found that glucose enhances BRI1 endocytosis to modulate BR signaling and thus alter the root growth direction (Singh et al., 2014).

BRs CONTROL NODULATION AND MYCORRHIZA FORMATION

Nodulation is a symbiotic process leading to the formation of nodules when Leguminosae roots are infected by rhizobia bacteria from soil. The bacteria in nodules convert atmospheric nitrogen to ammonium or nitrogen dioxide, which can be utilized for plant growth and development (Mylona et al., 1995). Because plants consume energy to form nodules, the number of nodules per plant is strictly controlled by specialized autoregulation of the nodulation pathway (AON pathway) (Reid et al., 2011). NODULATION AUTOREGULATION RECEPTOR KINASE (NARK) complexes with CLAVATA2 (CLV2) and/or KLAVIER (KLV) to control nodule numbers in roots (Miyazawa et al., 2010; Krusell et al., 2011; Foo et al., 2014). RDN1 is another important regulator in the AON pathway. Mutations of *NARK*, *CLV2*, *KLV*, or *RDN1* genes lead to a supernodulation phenotype (Miyazawa et al., 2010; Krusell et al., 2011; Foo et al., 2014).

BRs play essential roles in nodule formation. LK and LKB, a 5-α reductase and a C-24 reductase, respectively, were identified as BR biosynthetic enzymes in pea. *LKA* encodes the BR receptor in pea. Mutation of *LK*, *LKB*, or *LKA* resulted in reduced nodule organogenesis, indicating that BRs play important roles in regulating nodule numbers in pea (Ferguson et al., 2005). Double mutants generated from BR- and AON-related single mutants, such as *nark lk*, *clv2 lk*, and *rdn1 lk*, all showed a supernodulation phenotype similar to that of AON mutant lines, indicating that the AON pathway regulates nodule formation independent of BR (Foo et al., 2014). In soybean, however, exogenous application of BRZ enhanced nodule formation, suggesting that BRs function as an inhibitor of nodulation in soybean (Terakado et al., 2005). Nonetheless, additional studies are required to investigate why BRs play opposite roles in regulating nodulation in different species.

Arbuscular mycorrhiza formation is a widespread mutualistic symbiosis process between fungi and most families of land plants (Strack et al., 2003). Mycorrhizal plants benefit from their fungal partners by getting better access to soil nutrients, especially phosphorus, in nutrient-poor environments. In exchange, the fungi receive carbohydrates that are essential for their survival (Strack et al., 2003). So far, little is known about the roles of BRs in mycorrhizal symbiosis. Tomato d^X mutants that are defective in BR biosynthesis showed decreased mycorrhization, suggesting that BRs affect mycorrhizal infection and colonization (Bitterlich et al., 2014). The levels of various sugars, which supply enough energy for maintaining functional mycorrhiza, are reduced in the d^X mutant (Bitterlich et al., 2014). The sucrose transporter SlSUT2 interacts with BR biosynthesis and signaling components to regulate mycorrhizal symbiosis (Bitterlich et al., 2014).

INTERPLAYS OF BRs AND OTHER PHYTOHORMONES DURING ROOT GROWTH AND DEVELOPMENT

The components regulating root growth and development form a complex network. Besides BRs, other phytohormones are also known to control root growth and development. The central question is how these phytohormones interplay during these processes.

Crosstalk between BRs and auxin regulates various aspects of plant root growth and development (Kim et al., 2000; Bao et al., 2004; Li et al., 2005; Kim et al., 2007; Chaiwanon and Wang, 2015). First, auxin has been proven to be involved in BR biosynthesis. Auxin induces the expression of *DWF4* exclusively in roots, however, BRs inhibit *DWF4* expression by a feedback mechanism (He et al., 2005; Yoshimitsu et al., 2011). In addition, auxin-induced lateral root elongation was suppressed in a *dwf4* background or by BRZ treatment, which could be rescued by BL application (Yoshimitsu et al., 2011). This confirms that BRs and auxin oppositely regulate *DWF4* expression to control root growth. Second, BIN2 is a key component mediating BR and auxin signaling during root development. BR signaling inhibits BIN2 activity to activate BES1 and BZR1 transcription factors, leading to downstream regulation of plant growth and development (He et al., 2002; Wang et al., 2002; Yin et al., 2002). Auxin-mediated *DWF4* expression and auxin-caused increase in the number of lateral roots are elevated in *bin2*, a gain-of-function mutant, compared with wild-type or *bri1-5*, suggesting that BIN2 plays an important role in auxin signaling, especially during lateral root development (Maharjan et al., 2011). Yet, the mechanism of auxin-mediated BIN2 activation for lateral root development is largely unknown. Recent studies have found that BIN2 can also regulate lateral root organogenesis via phosphorylating ARF7 and ARF19 (Cho et al., 2014). Third, BRs participate in Aux/IAAs-mediated root development. Both *iaa7/axr2-1* and *iaa17/axr3-3* mutants that are insensitive to auxin showed increased BL sensitivities in roots (Nakamura et al., 2006). Further, exogenous application of BL can induce expression of some *Aux/IAA* genes that are involved in root development (Kim et al., 2006a). The above results imply the merging of BR signals into the auxin signaling pathway during root development. Fourth, as mentioned above, BRs control root growth by altering polar auxin transport and its distribution in roots (Bao et al., 2004; Li et al., 2005). Finally, recent studies suggested that BRs and auxin show opposite gradient patterns along the roots (Chaiwanon and Wang, 2015). They interact with each other both synergistically and antagonistically to control different aspects of root growth and development (Chaiwanon and Wang, 2015; Vragovic et al., 2015).

BIN2 mediates the interaction between BR and ABA in root development. It was found that ABA can repress both *DWF4* expression and lateral root development. This ABA inhibitory effect was lower in a *bin2* background, suggesting a role of BIN2 in ABA-mediated *DWF4* expression and lateral root development (Maharjan et al., 2011). Early studies have found that BR-deficient or signaling mutants, such as *cpd*, *det2*, and *bri1*, are hypersensitive to ABA in primary root inhibition (Clouse et al., 1996; Rodrigues et al., 2009). A recent study discovered that while *bin2-3 bil1 bil2* was less sensitive to ABA, *bin2-1* (gain-of-function mutation of BIN2) was hypersensitive to ABA in primary root inhibition (Cai et al., 2014). Subgroup III Snf1-related kinase 2s (SnRK2s) are crucial positive regulators in the ABA signaling pathway, responsible for ABA-regulated primary root elongation. Genetic and biochemical analysis demonstrated that BIN2 physically interacts with SnRK2 kinase and positively regulates ABA signaling to inhibit root elongation (Cai et al., 2014). However, *bes1-D* and *bzr1-1D*, dominant mutants with enhanced BR signaling outputs and acting downstream of BIN2, were also hypersensitive to ABA in primary root inhibition, suggesting BRs may also promote ABA signaling through downstream transcription factors (Rodrigues et al., 2009). The detailed molecular mechanism mediating such crosstalk between BR and ABA still needs further investigation.

Interactions between BRs and ethylene were reported for their regulation during root cell elongation. While targeted expression of *BRI1* in hair cells drives cell elongation in all tissues, its expression in nonhair cells inhibits root cell elongation. It was found that BRI1 activity in nonhair cells elevates expression of *ACS* genes. Consequently, the ethylene precursor ACC accumulates and enhances ethylene signaling, which inhibits unidirectional cell expansion (Fridman et al., 2014).

BRs negatively regulate the effect of jasmonic acid (JA) on *Arabidopsis* root growth. JA inhibits root growth in *Arabidopsis* (Browse, 2005). CORONATINE INSENSITIVE 1 (COI1), an F-box protein, functions as a receptor of JA (Xie et al., 1998; Chini et al., 2007; Thines et al., 2007). The root growth of a *coi1* mutant was insensitive to JA, which can be partially suppressed by a *dwf4* leaky mutant. Exogenous BL application attenuated JA inhibition of root growth, while BRZ treatment increased the function of JA (Ren et al., 2009; Huang et al., 2010).

CONCLUSIONS AND PERSPECTIVES

Root biology is essential for developing strategies to optimize agricultural land use and increase crop yield. Roots not only provide anchoring and mechanical support but also control nutrient and water uptake. Roots can also serve as important storage organs in some plant species and are sites of synthesis of important compounds required for optimal plant growth and development. Manipulating root systems to better locate and utilize nutrients existing in natural soil is a feasible strategy for producing high-yield crops in the near future.

Recent studies have greatly advanced our understanding of BR functions in root growth and development. First, BRs regulate root meristem size and lateral root development in a concentration-dependent manner. A low concentration of BRs promotes root growth, whereas a high concentration of BRs inhibit root growth (González-García et al., 2011; Hacham et al., 2011; Chaiwanon and Wang, 2015; Gupta et al., 2015; Lee et al., 2015). Second, BRs function in a cell-type-specific manner during root growth and development. BR signaling in the epidermis or in the inner tissues can trigger opposite effects on root meristem size (Hacham et al., 2011; Chaiwanon and Wang, 2015; Vragovic et al., 2015). BRs in H or N cells not only determine the epidermal cell fate but also affect root cell elongation (Cheng et al., 2014; Fridman et al., 2014). Third, BRs

interplay with other signals to either synergistically or antagonistically regulate root growth and development at various developmental stages (Kim et al., 2000; Bao et al., 2004; Li et al., 2005; Kim et al., 2007; Ren et al., 2009; Huang et al., 2010; Lanza et al., 2012; Chang et al., 2013; Bergonci et al., 2014; Singh et al., 2014; Gupta et al., 2015). Finally, BRs play important roles in regulating root nodulation and mycorrhiza formation (Terakado et al., 2005; Bitterlich et al., 2014; Foo et al., 2014).

Although great advances have been achieved in our understanding of BRs in regulating root growth and development, many questions remain unanswered. For example, why can a low concentration of BRs promote but a high concentration of BRs inhibit root growth and development? Low-affinity and high-affinity SA receptors were recently identified in plants (Fu et al., 2012). Are there any low-affinity BR receptors in roots that can sense and respond to high levels of BRs? Alternatively, if there is no low-affinity BR receptor, what is the molecular basis for a high concentration of BRs inhibiting root growth when applied exogenously? In addition, it remains unclear how plants can sense internal and external cues to control BR homeostasis. This is particularly important as many BR-regulated aspects of plant growth and development are often concentration dependent. Our understanding of BRs in regulating root growth, development, and interactions with root microorganisms is still at an early stage. Further studies are needed to understand detailed mechanisms of BRs in regulating these critical processes, which will benefit modern agriculture in the future.

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