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The brassinosteroid signaling network - a paradigm of signal integration

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Abstract

Many hormonal and environmental signals regulate common cellular and developmental processes in plants. While the molecular pathways that transduce these signals have each been studied in detail, how these pathways are wired into regulatory networks to provide the coordinated responses has remained an outstanding question. Recent studies of the brassinosteroid signaling network have revealed extensive signal integration through direct interactions between components of different signaling pathways. In particular, a circuit of interacting transcription regulators integrates many signaling pathways to enable coordinated and coherent regulation of seedling morphogenesis by hormonal and environmental signals. The recent studies support an emerging theme that complex networks of highly integrated signaling pathways underlie the high levels of developmental plasticity and environmental adaptability of plants.

Introduction

While discovered for its prominent role in promoting cell elongation, brassinosteroid (BR) actually regulates diverse developmental and physiological processes, including seed germination, seedling photomorphogenesis, stomata differentiation, organ boundary formation, flowering, male fertility, and plant responses to biotic and abiotic stresses [1-5]. In maize, BR also plays a role in sex differentiation [6]. Genetic and biochemical studies in Arabidopsis have elucidated in molecular details a complete BR signal transduction cascade from perception by the BRASSINOSTEROID INSENSITIVE1 (BRI1) receptor kinase at the cell surface to transcriptional regulation of thousands of nuclear genes by the BRASSINAZOLE RESISTENT (BZR) family transcription factors [1]. How this BR signaling pathway regulates the diverse developmental and physiological processes has

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become an outstanding question in the BR field. Likewise, many signaling pathways have been studied in detail, but separately, and how signaling pathways are integrated to ensure coordinated and coherent responses is becoming a prominent question in plant biology. Recent studies have revealed major mechanisms of BR crosstalk with other signaling pathway and the BR signaling network serves as a model for understanding the mechanisms of signal integration in plants.

BR signaling pathway: a brief account

According to the crystal structure reported recently [7,8], BR directly binds to the extracellular domain of BRI1 at a pocket formed by the folding of the island loop onto a region of the leucine-rich repeat (LRR) module, and this creates a surface for dimerization with the co-receptor kinase BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) or its homolog SOMATIC EMBRYOGENESIS RECEPTOR KINASE 1 (SERK1). As such BR acts as a “molecular glue” to bring together BRI1 and BAK1. Sequential transphosphorylation between the kinase domains of BRI1 and BAK1 activates the kinases, and BRI1 in turn phosphorylates members of two groups of plasma membrane-anchored cytoplasmic kinases, BRASSINOSTEROID-SIGNALLING KINASE1 (BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH1 (CDG1). CDG1, and likely also BSK1, phosphorylates the BRI1-SUPPRESSOR1 (BSU1) phosphatase, which then dephosphorylates and inactivates the GSK3-like kinase BRASSINOSTEROID INSENSITIVE2 (BIN2) [9]. When the BR levels are low, BIN2 phosphorylates two homologous transcription factors, BRASSINAZOLE RESISTANT1 (BZR1) and BZR2 (also named BRI1-EMS-SUPPRESSOR1, BES1), to inhibit their nuclear localization and DNA-binding activity. When the BR levels are high, BIN2 is inactivated, and BZR1 and BZR2 are dephosphorylated by PROTEIN PHOSPHATASE 2A (PP2A). Unphosphorylated BZR1 and BZR2 accumulate in the nucleus and bind to the promoters of target genes to confer BR-responsive gene expression [1].

BZR1 and BZR2/BES1 regulate overlapping sets of target genes, which include large numbers of genes with structural and metabolic functions such as cell wall biogenesis, as well as genes with regulatory functions, such as components of signal transduction pathways and key developmental regulators [10,11]. Although earlier studies of different target genes suggested opposite transcriptional activities, the genome-wide analysis showed that BZR1 and BZR2/BES1 regulate shared target genes in similar manner and they both activate and repress roughly equal numbers of target genes [10,11]. The transcriptional activities of BZR1 and BZR2/BES1 are modulated or mediated by interactions with other transcription factors and histone-modifying enzymes, such as the TOPLESS repressor and histone deacetylases [3,12,13]. BR can regulate specific developmental processes through BZR1 target genes, such as the *CUP-SHAPED COTYLEDON* (*CUC*) genes involved in organ boundary formation [4]. BR also regulates development through crosstalk mediated by direct interactions between the components of BR pathway and other signaling pathways. Below, we review recent progress in the studies of crosstalks between BR and other signaling pathways, focusing on the molecular mechanisms of direct interactions between components of different signal transduction pathways.

Integration of BR, auxin, and phytochrome pathways through interaction of BZR, ARF, and PIF factors

Seedling morphogenesis is controlled by a number of endogenous and environmental signals, including BR, auxin, gibberellin (GA), the circadian clock, light, and temperature. BR, and specifically the activity of BZR factors, is essential for seedling etiolation in the dark. BZR1/2 regulates light responses in at least two ways: it transcriptionally controls the expression levels of many light-signaling components, and it interacts with phytochrome-interacting factors (PIFs) [1]. The BZR1/2-mediated transcriptional repression of light signaling components, including phytochrome and light activated transcription factors *GATA2/4, BZR1–1D SUPPRESSOR1* (*BZS1*), and *GOLDEN2-LIKE 2* (*GLK2*) [10,11,14,15], contribute quantitatively to BR modulation of light sensitivity, but cannot explain the essential role of BR in etiolation in the dark, where photoreceptors and light signaling pathways are inactive. This essential role of BR in etiolation appears to be mediated by BZR1/2 as an essential partner of PIFs, which are basic helix-loop-helix (bHLH) transcription factors that accumulate in the dark or shade but become hyper-phosphorylated and degraded upon interaction with light-activated phytochromes [16]. BZR1 and PIFs are genetically interdependent for promoting cell elongation and etiolation, and they directly interact and co-regulate a large number of shared target genes to promote cell elongation and suppress photomorphogenesis [17]. Such a model of functional interdependence between BZR1/2 and PIFs provides a mechanistic explanation for the antagonistic relationship between BR and light signals in regulating seedling morphogenesis (Figure 1). This model also explains the essential roles of BR in plant responses to additional signals that modulate PIF levels. In addition to light, temperature and the circadian clock also control the levels of PIF proteins transcriptionally [18]. Transcriptional activation of *PIF4* expression is essential for the heat-induced hypocotyl elongation, and this function of PIF4 also requires BR and BZR activity [17] (Figure 1).

Auxin and BR are known to be interdependent and synergistic in promoting Arabidopsis hypocotyl elongation, and they induce highly overlapping transcriptional responses [19,20]. Auxin sensitivity, as well as auxin level, is modulated by light, temperature, and circadian rhythm, through PIFs [21]. While BZR1- and PIF-mediated transcriptional regulation of auxin biosynthetic and signaling genes have been observed [10,22], a recent study revealed more direct roles of PIF factors and BZR1 in auxin response [23]. Identification of genome-wide targets of ARF6 revealed over 50% overlaps between the ARF6 target genes and the targets of BZR1 or PIF4 [23]. BZR1 and PIF4 interact with ARF6 directly and enhance its binding to the shared promoters. Genetic analyses confirmed interdependent relationships between these factors in activating shared target genes and promoting hypocotyl elongation. The study supports a model that the Arabidopsis hypocotyl elongation is regulated through cooperative interactions among BZR1, ARF6, and PIF4 at the promoters of overlapping target genes, many of which encode cell wall proteins involved in cell expansion [23]. Thus, the BZR-ARF-PIF module elegantly explains the co-regulation of shoot cell elongation by BR, auxin, and phytochrome. Furthermore, additional signals regulate plant growth by modulating the activities of these factors.

Integration of gibberellin signal through DELLA repression of BZR, PIF, and ARF factors

GA binds to its receptor GID1 to induce ubiquitination and degradation of the DELLA proteins. Interestingly, DELLA proteins interact with and inhibit DNA binding activities of not only PIFs and BZR1, but also ARF6 [24-27]. Thus, GA promotes cell elongation largely by releasing the DELLA-mediated repression of PIFs, BZR1 and ARF6 [26-28]. GA/DELLA regulation of all three components of the BZR-ARF-PIF module potentially provides balanced and quantitative modulation of the outputs of the BZR-ARF-PIF circuit (Figure 1).

Integration of the strigolactone signaling pathway through MAX2-BZR interaction

Strigolactone (SL) suppresses lateral shoot branch growth and also promotes photomorphogenesis [29,30]. A recent study provided evidence that MORE AXILLARY GROWTH2 (MAX2), an F-box ubiquitin E3 ligase required for SL signaling, mediates ubiquitination and degradation of BZR proteins [31]. Yeast two-hybrid and *in vivo* assays showed MAX2 interaction with BZR1 and BZR2/BES1. SL treatment caused BZR2/BES1 degradation in a MAX2-dependent manner, and SL inhibition of hypocotyl elongation is abolished in both *max2* and the dominant *bes1-D* mutant. Consistent with PIF-dependent function of BZR in photomorphogenesis, the *pif-q* mutant is hypersensitive to SL [32]. Interestingly, the *bes1-D* mutant showed increased branching, and knockdown of *BES1* suppressed the more-axillary-growth phenotype of *max2*. It was proposed that the SL/MAX2-dependent degradation of BZR factors mediates SL regulation of both photomorphogenesis and axillary branch growth [31] (Figure 1).

Coupling of the BZR-ARF-PIF module with the tripartite HLH/bHLH module

The promotion of hypocotyl cell elongation by the BZR-ARF-PIF module requires a tripartite helix-loop-helix/basic-helix-loop-helix (HLH/bHLH) module consisting of two classes of non-DNA-binding HLH factors that antagonistically control many DNA-binding bHLH factors [26,33,34]. BZR1, ARF6, and PIF4 directly activate members of the PACLOBUTRAZOLRESISTANCE (PRE) family of HLH factors, which promote plant growth [26]. PREs bind to another group of HLH factors, including ILI1 binding bHLH1 (IBH1), LONG HYPOCOTYL IN FAR-RED (HFR), PHYTOCHROME RAPIDLY REGULATED1/2 (PAR1/2), and ATBS1 INTERACTING FACTOR (AIFs), which inhibit plant growth. Through heterodimerization, HFR and PAR1/2 inhibit DNA binding activities of PIFs [35,36]. As such, activation of *PRE* expression by the BZR-ARF-PIF module further increases availability of PIFs by sequestrating HFR and PAR1, forming a positive feedback loop. In contrast, activation of PIFs by shade conditions increases transcription of *HFR* and *PAR1* genes, forming a negative feedback loop. Such hormone-dependent positive feedback and hormone-independent negative feedback regulation of PIFs potentially ensures that the responses to shade or darkness are limited by the endogenous signals BR and auxin (Figure 1).

In addition to PIFs, PAR1 also inhibits BR-ENHANCED EXPRESSION 2 (BEE2), which is transcriptionally activated by BR signaling, and BIM1, which interacts with BZR2/BES1. Both BIM1 and BEE2 play a role in promoting hypocotyl elongation [37].

IBH1 inhibits another family of DNA-binding bHLH factors including HOMOLOG OF BEE2 INTERACTING WITH IBH1 (HBI1), BEE2 and three ACTIVATOR FOR CELL ELONGATION factors (ACE1 to ACE3) [33,34]. PRE1 binds to IBH1 to prevent its inhibition of HBI1 and ACEs [33,34]. Genome-wide target gene analyses showed that the HBI1 target genes are mostly (>70%) also direct targets of PIFs, and that HBI1 and PIFs activate many common target genes involved in cell elongation [38]. Like PIF4, HBI1 also interacts with ARF6[23]. But unlike PIFs, HBI1 is not regulated by light, and HBI1 positively regulates many genes encoding chloroplast proteins, suggesting a distinct role in promoting both growth and photosynthesis in light-grown plants [38]. On the other hand, expression levels of *HBI1* and *BEE* family members are repressed by growth-inhibition signals such as pathogen signals and abscisic acid (ABA) [38-40].

Integration with biotic and abiotic stress signals

BR and the pathogen associated molecular patterns (PAMPs) antagonistically regulate growth and immunity. Many molecular connections are known between the BR pathway and the pathway activated by the PAMP signal flagellin [41]. First, BRI1 and the flagellin receptor kinase FLAGELLIN-SENSITIVE2 (FLS2) share both co-receptor kinase BAK1 and the substrates BIK1 and BSK1 kinases [42,43] (Figure 2A). The functional significance of these upstream interactions in the BR-PAMP antagonism remains questionable, mostly because flagellin showed no effect on BZR1 phosphorylation and accumulation [44-46]. In contrast, significant crosstalk has been observed at the level of transcriptional regulation [38,39,47].

Both BZR1 and HBI1 have been shown recently to mediate transcriptional repression of immunity. BZR1 interacts with WRKY40 and activates the expression of several other WRKY transcription factors that inhibit immune responses [47]. However, the lack of a PAMP-induced effect on BZR1 level suggests that PAMP inhibition of BR-induced growth should be mediated by a component parallel or downstream of BZR1 [46]. Indeed transcription of *HBI1* is rapidly repressed by PAMP signals such as flagellin and elf18 [38,39]. Overexpression of *HBI1* significantly reduces the growth inhibition by flagellin and elf18, indicating that PAMP signaling inhibits growth mainly by repressing *HBI1*. Surprisingly, the *HBI1-overexpression* plants also show diminished PAMP-induced defense responses, including reactive oxygen species (ROS) production, defense gene expression, and resistance against pathogen infection [38]. The results demonstrate that HBI1 is activated by growth hormones at the protein level but repressed by PAMP signaling at the RNA level, and HBI1 in turn promotes growth and inhibits immunity. Therefore, HBI1 appears to function as a major node of crosstalk between the hormonal and PAMP signaling pathways [38,39] (Figure 1 and 2A).

Abiotic stresses activate production of ABA, which induces stress responses and inhibits plant growth. BR and ABA antagonize each other in many developmental processes, and various interactions between the two pathways have been observed at molecular levels. First, BR and ABA were shown to induce and repress, respectively, the expression of the BEEs, and genetic analysis supported a role of BEEs in the antagonistic interaction between BR and ABA [40]. Second, ABA was shown to increase phosphorylation of BZR1 [48].

Third, BIN2, the negative regulator of BR signaling, was recently shown to phosphorylate and activate the SnRK2 kinase, a positive regulator of ABA signaling [49]. Fourth, the BR-activated BES1 was shown to transcriptionally repress the ABA-signaling components ABI3 and ABI5 by recruiting the TOPLESS family of repressors [13], which appears to be a general mechanism of BR-induced transcription repression [12]. The relative contributions of these molecular mechanisms to the BR-ABA antagonism require further clarification. Genetic analyses have yielded conflicting results about the ABA-sensitivity phenotypes of the BR-hypersensitive *bes1-D* mutant [13,49].

Signal crosstalk with other receptor kinase pathways in specific developmental context

In addition to the flagellin/FLS2 pathway, several receptor kinase pathways have recently been shown to interact with the BR/BRI1 pathway, allowing BR regulation of specific differentiation and developmental processes.

The density and distribution of stomata at leaf surface are important for photosynthesis and water use efficiency. Stomata formation is negatively regulated by the ERECTA receptor kinase and a downstream MITOGEN-ACTIVATED PROTEIN (MAP) kinase module that inactivates the bHLH factor SPEECHLESS (SPCH), which promotes stomatal development [50]. BR inhibits stomatal development in leaves through BIN2-mediated phosphorylation and inhibition of the MAP kinase kinase kinase YODA and its substrate MAP kinase kinases (MKK4 and MKK5) [51,52]. BR also positively affects stomatal development in hypocotyl through inhibiting BIN2 phosphorylation and inactivation of SPCH [53]. Thus, BR signaling through BIN2 phosphorylation of several components of the ERECTA-MAPK-SPCH pathway inhibits and promotes stomata differentiation in different organs (Figure 2B). While BR inhibition of stomata formation in leaves is consistent with coordinated inhibition of photomorphogenic development, the biological significance of BR promotion of stomata formation in hypocotyl remains unclear. Whether ERECTA regulates the MAP kinases through BIN2 remains to be elucidated.

Two recent studies demonstrated that direct regulation of BIN2 by a receptor kinase involved in vascular tissue and lateral root development (Figure 2C). The tracheary element differentiation inhibitory factor (TDIF) is a peptide signal that activates the TDIF RECEPTOR (TDR) receptor kinase to inhibit xylem cell differentiation. In contrast, BR promotes xylem differentiation. Kondo et al showed that TDR kinase direct phosphorylates and activates BIN2, leading to phosphorylation and inactivation of BES1 [54]. As such, BR/BRI1 signaling pathway converges with the TDIF/TDR pathway at BIN2 in antagonistic regulation of xylem differentiation [54] (Figure 2C). In contrast, Cho et al, showed that, upon TDR activation, BIN2 phosphorylates the auxin response factor ARF7, to increase ARF7 activity and promote lateral root development. Intriguingly, BR promotes lateral root development independent of BIN2, whereas the TDR-BIN2-ARF7 module promotes lateral root development independent of BR signaling [55] (Figure 2C). How BIN2 performs both BR-dependent and BR-independent function remains unclear.

Conclusions

Integration of signaling pathways is crucial for the robustness of a regulatory system. Recent studies have demonstrated extensive integration of the BR signaling pathway with many other signaling pathways, through molecular interactions at levels of both signal transduction and transcriptional regulation. In particular, the BZR-ARF-PIF module coupled with the bHLH module appears to constitute a central growth regulation (CGR) circuit that integrates major hormonal and environmental signals. The CGR circuit not only reveals a central mechanism of cell elongation regulation but also provides an elegant example of how signaling pathways can be integrated to provide coordinated regulation of a common cellular response. It should be pointed out that the models presented in this review are likely overly simplified, due to space limit of the journal as well as incompleteness of our knowledge. The signal integration and outputs are likely modulated by tissue- and cell type-specific factors. In addition, the cross regulation of hormone levels is an integral part of the regulatory network that is not discussed in this review. A high degree of signal integration and high level of complexity of the growth-regulation networks is expected from the robustness and high plasticity of plant growth and development. A complete understanding of such networks will be important for improving plant productivity.

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•• of outstanding interest

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Highlights

- The BZR-ARF-PIF/DELLA module integrates major hormonal and environmental signals.
- BZR-ARF-PIF/DELLA module is coupled with the PRE-IBH1-HBI1 tripartite HLH/bHLH module.
- Biotic and abiotic stresses antagonize growth-promoting pathways at multiple levels.
- BIN2/GSK3 mediates crosstalks between BR and other receptor kinase pathways.
- The TDR receptor kinase phosphorylates BIN2 to regulate xylem and lateral root development.

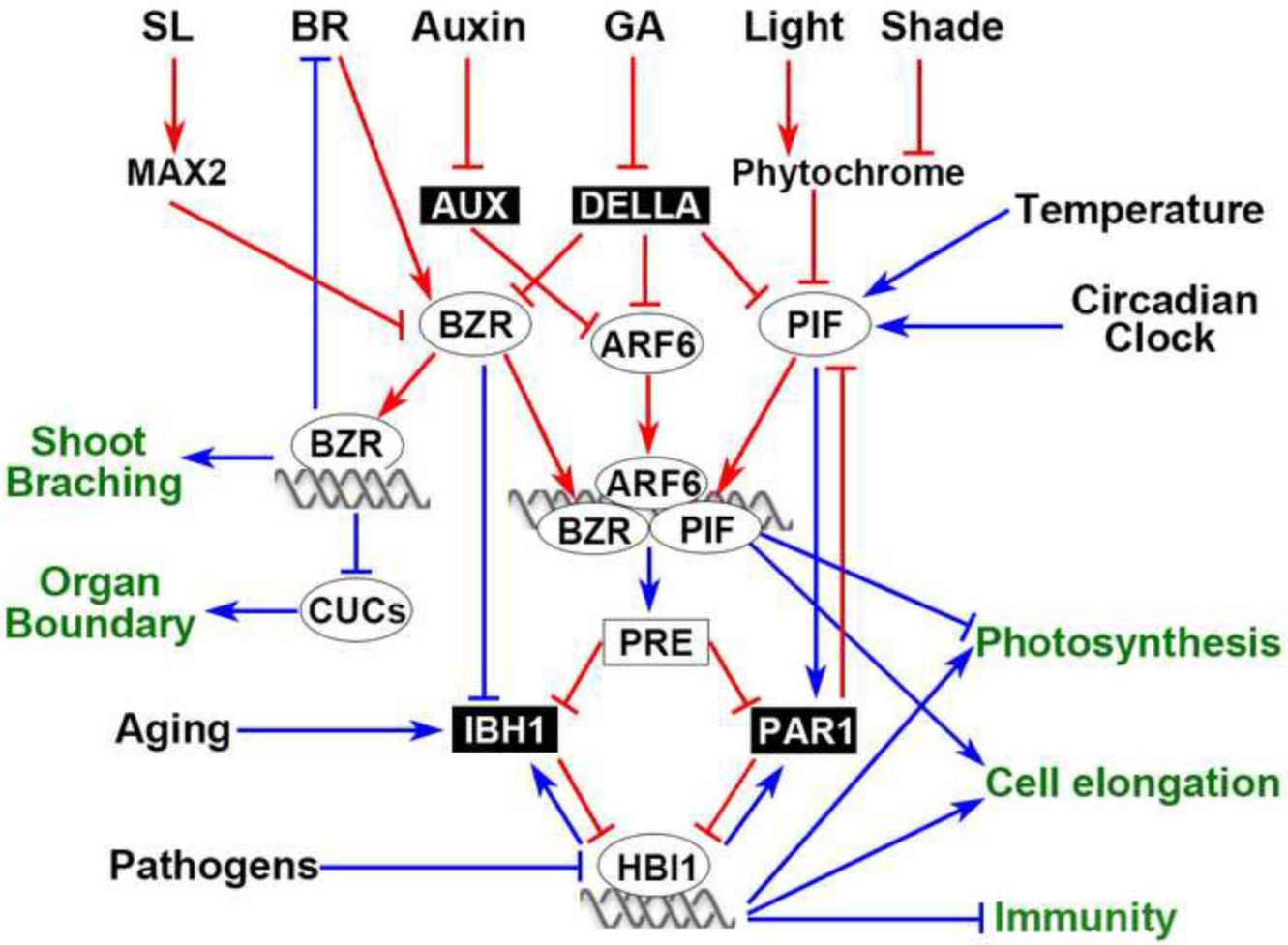

Figure 1.

A central growth regulation network integrates hormonal and environmental signals in transcriptional regulation of plant growth and physiology. DNA-binding transcription factors are shown in ovals, and non-DNA-binding factors that inhibit DNA binding factors are shown in black boxes. Red lines show posttranslational activation (arrows) or inhibition (bar end), and blue lines show transcriptional activation (arrows) or inhibition (bar end).

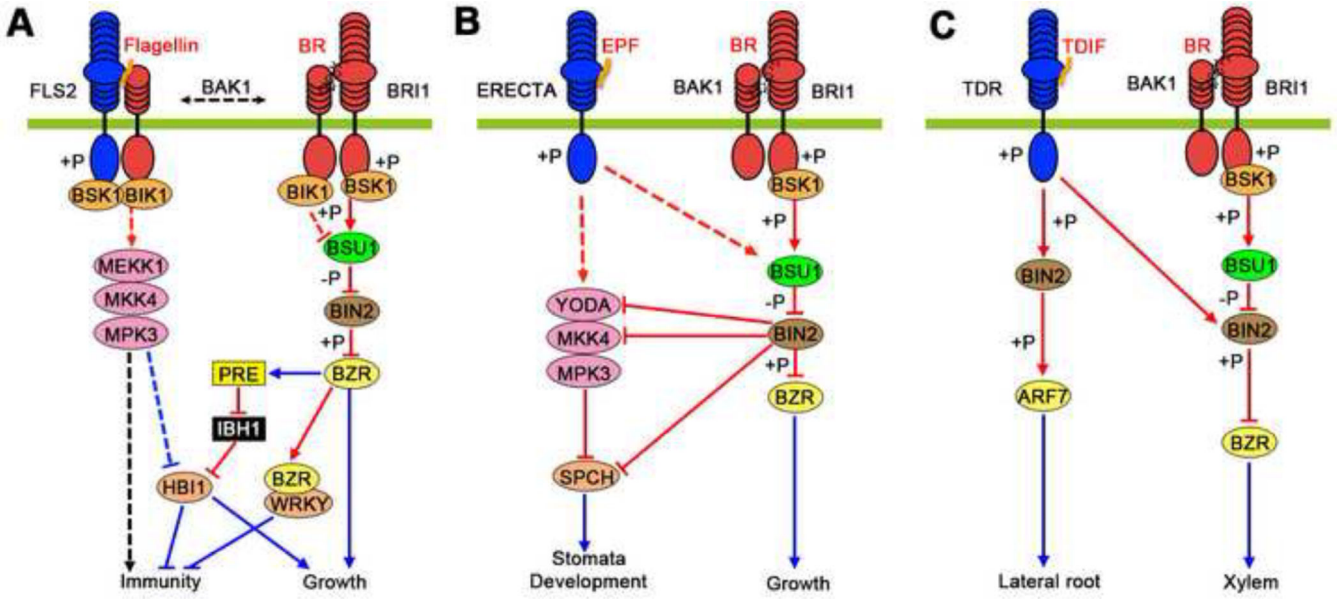

Figure 2.

Crosstalks between BR/BRI1 and other RLK pathways.

A. Crosstalk between BR/BRI1 and flagellin/FLS2 pathways regulating the tradeoff between growth and immunity.

B. BR/BRI1 crosstalks with the ERECTA-MAPK pathway to regulate stomata development.

C. BR/BRI1 crosstalk with the TDIF/TDR pathway to antagonistically regulate xylem differentiation. TDIF/TDR also regulates BIN2 to promote lateral root development, independent of BR signaling.

Red lines show posttranslational activation (arrows) or inhibition (bar end) by phosphorylation (+p) or dephosphorylation (-p), and blue lines show transcriptional activation (arrows) or inhibition (bar end).