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REVIEW

The brassinosteroid signal transduction pathway

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Steroids function as signaling molecules in both animals and plants. While animal steroid hormones are perceived by nuclear receptor family of transcription factors, brassinosteroids (BR) in plants are perceived by a cell surface receptor kinase, BRI1. Recent studies have demonstrated that BR binding to the extracellular domain of BRI1 induces kinase activation and dimerization with another receptor kinase, BAK1. Activated BRI1 or BAK1 then regulate, possibly indirectly, the activities of BIN2 kinase and/or BSU1 phosphatase, which directly regulate the phosphorylation status and nuclear accumulation of two homologous transcription factors, BZR1 and BES1. BZR1 and BES1 directly bind to promoters of BR responsive genes to regulate their expression. The BR signaling pathway has become a paradigm for both receptor kinase signaling in plants and steroid signaling by cell surface receptors in general.

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Introduction

Since the first discovery of the growth promoting activity in extracts of rape pollen in 1970 [1] and the identification of the bioactive compound brassinolide (BL) in 1979 [2], many plant steroids with similar structure and functions to BL have been identified throughout the plant kingdom, and they are defined as brassinosteroids (BRs). Physiological studies have demonstrated that BR can induce diverse cellular responses such as stem elongation, pollen tube growth, leaf bending and epinasty, root inhibition, induction of ethylene biosynthesis and fruit ripening, and xylem differentiation [3-6]. The identification of *Arabidopsis* BR biosynthetic mutants established that BR is a new class of phytohormone with an essential role in plant growth and development [7-9]. Since then, extensive molecular genetic and biochemical studies of BR signaling in *Arabidopsis thaliana* have illustrated a BR signal transduction pathway from ligand perception on the cell surface to gene expression in the nucleus. This review focuses on recent advances in our understanding of the mechanisms of BR perception, receptor kinase activation and BR regulation of gene expression, with highlights of outstanding questions in the field.

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BR is perceived by the BRI1 receptor kinase on cell surface

The brassinosteroid insensitive 1 (*bri1*) mutant was first identified about 10 years ago [10]. Additional alleles of *bri1* were isolated in subsequent screens for mutants with similar phenotypes to BR biosynthetic mutants but cannot be rescued by the application of BR [11]. Cloning of *BRI1* revealed that it encodes a leucine-rich repeat receptor-like kinase (LRR-RLK), containing an extracellular domain, a transmembrane domain and a cytoplasmic serine/threonine kinase domain. The extracellular domain of BRI1 was initially predicted to contain a leucine-zipper motif, 25 tandem LRRs, 2 cysteine pair before and after the LRRs, and a 70 amino acid (70 aa) island inserted between LRR21 and LRR22 [11, 12]. A recent reannotation of BRI1 predicted no leucine zipper and 24 rather than 25 LRRS, with LRR21 being an unusual methionine-rich repeat [13]. The intracel-

lular region was subdivided into a juxtamembrane region (JM), kinase domain, and a short C-terminal extension [13]. Mutations in large numbers of *bri1* alleles cluster in two regions, the intracellular kinase domain and the 70 aa island domain, suggesting important functions for these two regions [11, 12].

The structure of BRI1 and its essential role in BR response supported a popular hypothesis that BRI1 is the BR receptor. Evidence supporting this hypothesis has been obtained in several studies over the years and the final proof has been achieved recently. First, when the extracellular LRR and transmembrane domains of BRI1 were fused to the serine/threonine kinase domain of Xa21, a rice LRR receptor kinase for disease resistance, the chimeric receptor was able to elicit defense responses in rice cells upon treatment with BL [14]. Secondly, BRI1 was co-immunoprecipitated with the BR-binding activity and BR treatment was shown to induce BRI1 autophosphorylation, which indicates that BR interacts with BRI1 and activates its kinase activity [15]. However, these studies failed to demonstrate direct binding of BR to BRI1. In fact several lines of evidence supported the possibility that BRI1 might interact with another protein or peptide that binds directly to BR. For example, a secreted serine carboxypeptidase encoded by the *BRS1* gene was shown to play an important role in the early stage of the BR signaling and was thus proposed to process an extracellular protein/peptide involved in BR perception [16]. Furthermore, there are at least two putative steroid-binding proteins (SBPs) in the *Arabidopsis* genome, raising the possibility that BR might bind a SBP that interacts directly with BRI1 [17]. Although the possibility of BRI1 interacting with extracellular proteins cannot be excluded, a recent study from Joanne Chory's lab demonstrated that BR directly binds to the extracellular domain of BRI1 [18]. Using biotin-tagged photoaffinity castasterone (BPCS), a biosynthetic precursor of brassinolide with partial activity, they demonstrated that BR directly binds to BRI1 in Arabidopsis and to recombinant BRI1. BR binding assays using recombinant BRI1 fragments indicated that the minimal BR binding region in BRI1 consists of the 70-aa island and the carboxy-terminal flanking LRR (ID-LRR22). BR also binds specifically to the ID-LRR22 domain from BRL1 and BRL3, homologs of BRI1 that function as BR receptors in vascular differentiation [19, 20]. These studies unambiguously demonstrate that BRI1 is the bona fide BR receptor [18].

BR activation of BRI1 and BAK1 receptor kinases

BR binding activates the BRI1 kinase and causes autophosphorylation of BRI1, as demonstrated by both mobility shift of BRI1 in SDS-PAGE and by increased signal detected by anti-phospho-serine/threonine antibodies in BR treated samples [15, 21]. Many phosphorylation sites of BRI1 have recently been identified by mass spectrometry analysis of recombinant BRI1 expressed in *E. coli* or BRI1 immunoprecipitated from plant extracts [21, 22]. Mutation studies of the phosphorylation residues demonstrated that some of the phosphorylation events increase and some reduce the BRI1 activity, as measured by the ability of the mutated BRI1 protein to phosphorylate a synthetic substrate and to rescue a *bri1* mutant [21]. For example, mutations of Ser or Thr residues in the activation loop and the juxtamembrane and C-terminal domains reduced the kinase activity, whereas mutation of T872A increased the kinase activity [21].

BRI1 has been found to interact with another LRR receptor-like kinase named BAK1 (BRI1 associated receptor kinase 1), which was identified as a component of the BR signaling pathway by two independent groups based on its interaction with BRI1 kinase domain in yeast [23] and suppression of a weak *bri1* allele by BAK1 overexpression [24]. An obvious structural difference between BRI1 and BAK1 is that BAK1 has only five extracellular LRR repeats and no BR binding domain. Loss-of-function mutation of BAK1 caused a weak dwarf phenotype. However, overexpression of a dominant negative mutant form of BAK1 caused a severe dwarf phenotype similar to strong *bri1* mutants, suggesting that BAK1 plays a major role in transducing the BR signal and the weak phenotype of *bak1* mutant is likely due to redundant functions of its homologs [24]. *In vivo* interaction between BAK1 and BRI1 has been demonstrated by co-immunoprecipitation assays and fluorescence life time imaging microscopy [23-25], and it has been shown recently that BR treatment increases the BRI1-BAK1 interaction [21].

BR induced BRI1/BAK1 oligomerization and kinase activation suggests a mechanism of ligand-induced receptor kinase activation similar to those in animals. Studies of receptor kinases in animal systems have shown that activation often involves ligand-induced receptor dimerization or oligomerization, which causes kinase activation by transphosphorylation. For example, response to the transforming growth factor β (TGF-β) is mediated by two receptor serine/threonine kinases Tβ-RI and Tβ-RII. Tβ-RII homodimerizes in the absence of ligand and exhibits constitutive autophosphorylation [26]. TGF-β binding to Tβ-RII induces formation of the heterotetramer with Tβ-RI and phosphorylation of Tβ-RI by Tβ-RII [27]. Once activated by phosphorylation, Tβ-RI propagates the signal by phosphorylating cytoplasmic substrates that translocate to the nucleus to regulate the expression of TGF-β–responsive genes. In other cases, such as the EGF receptor kinases, monomeric receptors exhibit basal level or no kinase ac-

tivity, and ligand-induced receptor dimerization leads to transphosphorylation that activates the kinase. Models of BRI1 activation based on either TGF-β or EGF receptors have been proposed [23, 24]. In addition, it has been shown that the plant homolog of a mammalian Tβ-RII substrate is a putative BRI1 substrate in *Arabidopsis* [28], which provides further evidence for the similarity between TGF-β and BR signaling.

Although BR induced BRI1/BAK1 dimerization and kinase activation share similarities with animal receptor kinases, it is not fully understood whether dimerization of BRI1/BAK1 is the cause or result of ligand-induced activation of BRI1 kinase. Analyses of the effects of mutations that abolish kinase activity on the dimerization and transphosphorylation performed in yeast and *in vitro* yielded slightly conflicting results. Wild type BRI1 and BAK1 co-expressed in yeast interact with each other and show kinase activity. But when wild type BRI1 or BAK1 was co-expressed with a mutant BAK1 or BRI1, respectively, the receptors still interact with each other but no phosphorylation was detected for either wild type or the mutant kinase, suggesting that kinase activation, but not heterodimerization, requires kinase activities of both BRI1 and BAK1 [23]. In contrast, *in vitro* assays using the kinase domains expressed in *E. coli* showed that wild type BRI1 or BAK1 kinases can autophosphorylate as well as transphosphorylate mutant BAK1 or BRI1, respectively. Furthermore, the affinity for the interaction between BRI1 and BAK1 kinases is reduced by mutation of either kinase, with mutation of BRI1 kinase showing stronger effect than mutation of BAK1. The *in vitro* data is thus consistent with the hypothesis that BR activation of BRI1 kinase activity leads to dimerization with and subsequent activation of BAK1 [24].

The second hypothesis is supported by a recent study from Joanne Chory's lab, which provided evidence that receptor activation involves ligand induced conformation changes in the BRI1 kinase [29]. In this study, deletion of the C-terminal 40 amino acids (CT) of BRI1 was shown to increase receptor activity, as indicated by increased phosphorylation of BRI1 and growth response in transgenic plants expressing such truncated BRI1 receptor [29]. The inhibitory function of the CT region appears to be reduced by phosphorylation, because the kinase activity was increased by mutation of several Ser/Thr residues in this region to Asp, which likely mimics phosphorylation [29], and reduced by mutations of Ser/Thr to Ala, which prevent phosphorylation [21]. BRI1 was also shown to interact with itself *in vivo* and the interaction appears to be increased by BR, suggesting that BR binding stabilizes the BRI1 homodimer. Based on these observations, it was proposed that homodimerization is involved in BRI1 kinase activation [29]. In the absence of ligand, BRI1 exists as a preformed inactive homo-oligomer, and its kinase activity is maintained at a basal level via *trans*- or *cis*-repression by its CT domain. Steroid binding to the extracellular domain of BRI1 induces a conformational change in the kinase domain and allows trans-phosphorylation of the CT region, which likely enhances BRI1's kinase activity and promotes further phosphorylation of BRI1, thereby providing binding sites for BAK1 [29].

Although the model of Wang *et al*. [29] is consistent with most of the observations, many questions remain to be answered. For example, the possibility that activation of BRI1 kinase requires dimerization with BAK1 has yet to be tested experimentally. BR binding to BRI1 might induce conformational changes in either the extracellular or kinase domain that facilitate interaction with BAK1, and BAK1 might then further activate BRI1 kinase through transphosphorylation. Such a possibility is supported by the ability of BAK1 to phosphorylate mutant BRI1 kinase *in vitro* [24]. It would be interesting to test whether mutants lacking BAK1 and its homologs or the transgenic plants overexpressing the dominant negative *bak1* [24] show BR-induced BRI1 phosphorylation. Furthermore, the function of the extracellular domain of BAK1 has yet to be explained if interaction with BRI1 is only mediated by the intracellular kinase domains. The extracellular domain of BAK1 is apparently not involved in BR binding, because BR binding activity is not affected in the *bak1* null mutant [18] or transgenic plants overexpressing BAK1 [29]. However, the dominant negative effects of kinase-inactive mutants of BAK1 [24] and BRI1 [29] suggest that the receptors can interact with their partners independent of the kinase activity. Therefore, it is possible that the extracellular domain of BAK1 binds to the BR-occupied extracellular domain of BRI1 to initiate the heterodimerization. It would be interesting to test whether kinase-inactive mutant BRI1 and BAK1 can interact with each other in a BR-dependent manner in plant cells. The precise sequence of events in BR-induced receptor oligomerization and kinase activation remains to be further defined.

In addition to BAK1, two other putative BRI1 substrates have been identified. Transthyretin-Like (TTL) is an *Arabidopsis* protein with sequence similarity to vertebrate transthyretin [30]. TTL interacts with BRI1 in a yeast two-hybrid assays and the interaction is abolished by mutations that knockout the BRI1 kinase activity. Genetic studies suggested that TTL plays a negative role in BR responses. TTL is localized on the plasma membrane, though it contains no obvious transmembrane domain [30]. The function of TTL in BR signal transduction remains unclear [30]. AtTRIP1 is an *Arabidopsis* homolog of the mammalian TGFβ receptor interacting protein 1 (TRIP1).

In mammalian, TRIP1 is a cytoplasmic substrate of the TGFβ receptor TβII and functions as a subunit of the eIF3 translation initiation factor. BRI1, but not BAK1, can phosphorylate AtTRIP1 *in vitro* and interact with AtTRIP1 *in vivo*. It was proposed that AtTRIP1 might play a role in BR regulation of protein synthesis or in transducing BR signal to downstream components [28].

Tissue specific functions of the homologs of BRI1 and BAK1

BRI1 and BAK1 belong to a large family of plant-specific LRR-RLKs, consisting of more than 200 members in *Arabidopsis* and over 300 in rice [31]. Three close homologs of BRI1 have been characterized [19, 20, 32]. BRL1 and BRL3 bind BR with high affinity and VH1 showed very weak BR binding [18]. Genetic analysis suggests that they play a role in vascular development [19, 32]. BRI1, BRL1, and BRL3 have partially redundant function in BR signaling, and their functional specificities appear to be conferred by their tissue specific promoters rather than specific signaling function of the proteins, because expression of BRL1 and BRL3 using the BRI1 promoter can rescue the *bri1* mutant [19] and overexpression of BRL1 can partially suppress *bri1-5* [20].

BAK1 and four close homologs have been identified as somatic embryogenesis receptor kinases (SERKs) [33] and proposed to play a role in embryo development. A recent study showed that single knockout mutant of either SERK1 or SERK2 shows no obvious phenotypes, but double mutants are male sterile due to defect in tapetum differentiation, a phenotype similar to that caused by mutation of another LRR-RLK, *ems1* [34]. These results suggest that SERK1 and SERK2 are functionally redundant in reproductive development [35]. It is unclear whether the function of SERK1 and SERK2 in reproductive development is related to BR signaling. Strong BR-deficient and BR-insensitive mutants are also male sterile, although it remains to be analyzed if they have similar defect in tapetum differentiation. SERK1 was recently shown to be part of a protein complex that includes BRI1 and BAK1 [36], suggesting that SERK1 might play a role in BR signaling. How members of BRI1 and BAK1 families pair up in regulating various developmental processes and whether there exist tissue-specific BR signaling mechanisms are interesting questions to be answered in future studies.

Mutations of BRI1 orthologs in rice, pea, and tomato cause BR insensitive dwarf phenotypes [37-39], suggesting highly conserved function of BRI1. Interestingly, the tomato BRI1 was shown to function as receptor for both BR and systemin, a small peptide involved in plant defense (reviewed by Wang and He, 2004 [40]). It is currently not known how tomato BRI1 mediates two distinct responses and whether similar peptide ligand of BRI1 exists in *Arabidopsis* and other plants. Although the function of BRI1 as BR receptor is conserved in *Arabidopsis*, rice, pea and tomato, the functions of BRI1 homologs in regulation of developmental processes might be different in different species, as suggested by a recent functional study of the rice BRI1 gene family [41].

Downstream components of the BR signal transduction pathway

BR activation of BRI1 and BAK1 kinases initiates a signaling cascade that leads to nuclear gene expression. Downstream components of this cascade include the BIN2 kinase, the BSU1 phosphatase, and the transcription factors BZR1 [42, 43] and its homolog BES1/BZR2 [44, 45]. BZR1 and BES1/BZR2 are substrates of the BIN2 kinase [44, 46] and BSU1 phosphatase [47], and phosphorylated BZR1 is degraded by the proteasome [46]. In the absence of BR, BRI1 and BAK1 are inactive, and BIN2 phosphorylates BZR1 and BES1/BZR2 and targets them for degradation by the proteasome. BR induces dephosphorylation and accumulation of BZR1 and BES1/BZR2, possibly by inhibiting the BIN2 kinase or activating the BSU1 phosphatase. BZR1 and BES1 bind to the promoters of BR regulated genes to activate or repress gene expression [45, 46]. With direct interactions demonstrated for BR, BRI1 and BAK1, and among BIN2, BZR1/BES1 and promoter DNA, the only gap in the BR signal transduction pathway is between BRI1 or BAK1 and BIN2 or BSU1.

The BIN2 kinase phosphorylates BZR1 and BES1 to target them for degradation by the proteasome

BIN2 shares about 70% sequence similarity with the Drosophila shaggy kinase and mammalian glycogen synthase kinase-3 (GSK-3) in the catalytic domain [48]. In animals, GSK3/SHAGGY-like kinases play a key role in the regulation of many cell functions, including signaling by insulin, growth factors and nutrients, and the control of cell division and differentiation, apoptosis, and microtubule dynamics [49]. In these pathways, GSK3 functions as a negative regulator that reduces the activity of downstream substrate through inhibiting their biochemical activities, changing their subcellular localization, or promoting their degradation. Phosphorylation by GSK3 often requires prephosphorylation of the substrate at a priming site by other kinases. Signaling by cell surface receptors inhibits GSK3 through several mechanisms, including phosphorylation at the N terminal region of GSK3, preventing priming phosphorylation of its substrate that is required for phos-

phorylation by GSK3 , and disruption of protein complex of GSK3 and its substrate [50].

Like animal GSK3 kinases that inhibit the activity of their substrates, BIN2 functions as a negative regulator in BR signal transduction. Dominant mutations of *bin2* that increase the kinase activity or overexpression of the wild type BIN2 gene causes *bri1*-like BR insensitive phenotypes [52-54], which is associated with increased phosphorylation and reduced accumulation of BZR1 and BES1 [44, 46]. BIN2 interacts with and phosphorylates BZR1 and BES1 in yeast and *in vitro*. Phosphorylated BZR1 is stabilized by MG132, an inhibitor of the proteasome, suggesting that BIN2 phosphorylation leads to degradation of BZR1 by the proteasome. The large number of putative GSK3 phosphorylation sites in BZR1 and BZR2 sequence and the severe mobility shift caused by BIN2 phosphorylation suggests that BIN2 phosphorylates the substrates at multiple amino acid residues [42, 46]. The positions of these phosphorylation sites and their effects on BZR1/BES1 accumulation and activity are yet to be determined experimentally.

Unlike animal GSK3 kinase, which requires priming phosphorylation of its substrate, BIN2 relies on a direct kinase-substrate docking interaction to phosphorylate BES1 [55]. Plant GSK3 kinases also lack the N-terminal Ser residue that is conserved in animal GSK3 kinases and that inhibits kinase activity when phosphorylated [49]. Further studies will reveal whether BR signaling inhibits BIN2 kinase through a mechanism similar to those found in animal GSK3 kinases.

The BSU1 phosphatase dephosphorylates BES1

BR treatment induces rapid disappearance of the phosphorylated BZR1 and BES1, possibly due to either degradation by the proteasome or dephosphorylation by a phosphatase. Because BR-induced disappearance of phosphorylated BZR1 is not affected by MG132, a proteasome inhibitor that stabilizes phosphorylated BZR1 in the absence of BR treatment, it was proposed that a phosphatase is involved in BZR1 dephosphorylation [46]. Indeed a phosphatase with Kelch-repeat, BSU1, was shown to promote dephosphorylation of BES1. BSU1 was identified as an activation tagged suppressor of *bri1-5*, and overexpression of BSU1 increased cell elongation and the level of unphosphorylated BES1. Thus BSU1 appears to counteract the activity of BIN2 in regulating phosphorylation status of BES1 and likely also BZR1. *In vitro*, recombinant BSU1 was able to dephosphorylate BES1 that had been pre-phosphorylated by BIN2, but with very low efficiency. BSU1 did not directly interact with BES1 *in vitro*, and it was proposed that an adaptor protein might be required for BSU1 to effectively dephosphorylate BES1. BSU1 is constitutively localized in the nucleus, consistent with its action on BES1. It is unclear whether BSU1 activity is regulated by BR signaling [47].

BZR1 and BES1 directly regulate BR responsive gene expression

A genetic screen for mutants resistant to brassinazole lead to the identification of the *brassinazole resistant 1-1D* (*bzr1-1D*) mutant, which turned out to suppress the *bri1* and *bin2* mutants [42]. BZR1 encodes a nuclear protein and a P234L mutation in *bzr1-1D* stabilizes the protein. Same mutation in BZR1's closest homolog, BZR2/BES1, was later identified in a *bri1* suppressor screen [44]. BZR1 and BES1/BZR2 show 88% sequence identity and their phosphorylation and accumulation are similarly regulated by BR and BIN2 [42, 44, 46]. While both *bzr1-1D* and *bes1-D* suppress *bri1* and *bin2* and are resistant to brassinazole, they show opposite cell elongation phenotypes when grown in the light. The *bzr1-1D* plants grown in the light show weak dwarfism with reduced hypocotyl and petiole lengths, while *bes1-D* has elongated organs [42, 44]. The *bzr1-1D* plants contain reduced level of BR due to increased feedback inhibition of BR synthesis, and its weak dwarf phenotype can be rescued by BR treatment. It has been proposed that BZR1 plays dual roles in mediating feedback inhibition of BR biosynthesis and BR promoted growth responses [43].

The biochemical functions of BZR1 and BES1 have been illustrated recently [43, 45]. Through extensive protein-DNA interaction studies, including gel shift competition assays, DNA footprint, binding site selection from random sequences, and chromatin immunoprecipitation, He et al. demonstrated unequivocally that BZR1 specifically binds to CGTG(T/C)G sequence, which was named BR response element (BRRE) [43]. BRRE was found enriched in promoters of BR-repressed genes, including *CPD*, *DWF4*, and a large number of BR repressed genes identified in microarray studies. A BZR1-GAL4 fusion construct repressed the expression of a reporter gene containing GAL4 binding sites. Using chromatin immunoprecipitation, they showed that BZR1 binds to *CPD* and *DWF4* promoters *in vivo* and the binding was increased by BR treatment. Furthermore, BRRE was shown to be essential for BR regulation of the *CPD* promoter, because mutation of the BRRE in the CPD-GUS reporter gene abolished its repression by BZR1. These results demonstrate that BZR1 binds to the BRRE in BR responsive promoters to regulate their expression, thereby inhibiting BR biosynthesis as well as promoting growth responses [43]. It was proposed that BZR1 might indirectly activate BR-induced genes by repressing other transcription repressors [43], which is consistent with mi-

croarray data showing that most BR-induced genes respond slowly to BR treatment [56].

BES1 has also been shown to bind to DNA [45]. In this case, BES1 was shown to interact with three homologous basic-helix-loop-helix (bHLH) transcription factors (BIM1-3) in yeast two-hybrid assays [45]. Gel shift assays demonstrated that BES1 could bind to the E-box (CANNTG) elements in the promoter of SAUR-AC1, a gene showing early induction by both BR and auxin [56, 57]. The BIM1 protein was also shown to bind the same E-box, and synergism between BES1 and BIM1 was observed for both DNA binding *in vitro* and activation of SAUR-AC1 promoter in transient assays. Overexpression of BIM1 partially suppressed a weak *bril* allele, and triple mutant of *BIM1-3* showed reduced cell elongation, suggesting a role of the BIM1-3 proteins in mediating BR responses. It was proposed that BES1 and the BIM1-3 proteins synergistically bind to E-box elements to activate BR-induced gene expression [45] .

It is surprising that BZR1 and BES1 showed different DNA-binding specificities and opposite transcriptional activities, given their high sequence homology (88% overall amino acid sequence identity). Within the 83 amino acid region of BZR1 that is sufficient to bind DNA [43], only six amino acids differ between BZR1 and BES1, and only two of these six are in the N-terminal most conserved 69 amino acid region containing the predicted HLH domain [45]. It is possible that BZR1 and BES1 have similar DNA binding specificities. Although different DNA motifs were shown to bind to BZR1 and BES1, the binding specificities may not be as distinct as it appears. The BRRE element (CGTGTG) differs from the E-box (CANNTG) by only one base pair. In fact one of the mutated BRREs used in competition gel shift assays (m8, CATGTG) matches perfectly the E-box sequence [43]. However this mutated BRRE showed the weakest binding to BZR1 among all the DNAs with single nucleotide mutation of the core sequence, though it showed better binding than the one with all six base pairs mutated [43]. Therefore BZR1 is likely to discriminate against E-box among sequences similar to BRRE. On the other hand, whether BES1 binds BRRE remains unknown. In fact, the optimal binding site sequence of BES1 has yet to be determined, for example, by binding site selection from random sequences. Without the optimal binding site sequence for BES1 or direct comparison of E-box with BRRE for their binding affinities to BES1, it is difficult to conclude whether BES1 and BZR1 indeed have different DNA-binding specificity.

Is BZR1 a transcription repressor while BES1 an activator, or can they both activate and repress different promoters? Although BZR1 has been shown to repress the *CPD* promoter and BES1 to activate the *SAUR-Ac* promoter, it is not clear if the different transcriptional activities are due to different promoters used in the studies or intrinsic differences between BZR1 and BES1 proteins. The reduced *CPD* and *DWF4* expression in the *bes1-D* mutant [13, 47] suggests that BES1 might function as a transcription repressor on these promoters, possibly acting through BRRE. It is also possible that BZR1 might function as an activator for some BR induced genes, although BZR1 can repress transcription of BR down regulated genes and an artificial promoter [43]. The opposite cell elongation phenotypes of light-grown *bzr1-1D* and *bes1-D* are consistent with opposite transcriptional activities of the BZR1 and BES1 proteins, but such difference in phenotype could also be caused by different tissue-specific or light-regulated expression of the *BZR1* and *BES1* genes. Indeed transgenic plants overexpressing the mutant *bzr1-1D* gene show a long hypocotyl phenotype similar to *bes1-D* (Y Sun and Wang, unpublished data). Further studies will clarify the similarities and differences between BZR1 and BES1 at the biochemical and physiological levels.

Conclusion and prospect

Our understanding of BR signal transduction has greatly improved in recent years. The demonstration of BR perception by BRI1 and BR-induced BRI1-BAK1dimerization, the identification of phosphorylation sites and an autoregulatory domain of BRI1, and the discovery of DNA binding activities of BZR1 and BES1 have established the BR pathway as one of the best-understood signal transduction pathways in plants. Yet there are still many questions to be answered. The major gap in our knowledge of the BR signaling cascade is between BRI1/BAK1 and BIN2. The mechanisms by which BR signaling regulates BIN2 kinase and the BSU1 phosphatase will be a focus of future studies. In addition, the components that regulate the degradation or localization of phosphorylated BZR1 and BES1 are yet to be identified. While genetic studies have been fruitful in identifying the major components of the pathway, biochemical approaches are likely to play an increasingly important role in revealing the details and filling the gaps of the BR signaling pathway in future studies.

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