BBA - Gene Regulatory Mechanisms 1861 (2018) 561–571

Contents lists available at ScienceDirect

BBA - Gene Regulatory Mechanisms

journal homepage: www.elsevier.com/locate/bbagrm

Review

The brassinosteroid-regulated transcription factors BZR1/BES1 function as a coordinator in multisignal-regulated plant growth

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ARTICLE INFO

Keywords:
BZR1/BES1
Brassinosteroid
Signaling crosstalk
Transcriptional regulation
Protein-protein interaction
Plant growth and development

ABSTRACT

BZR1 and BES1 are key transcription factors of brassinosteroid (BR) signaling and represent the integration node of numerous signaling cascades. Their direct target genes have been identified, and BZR1/BES1-DNA interactions have been experimentally verified. Importantly, BZR1/BES1 also integrate different growth and development events via direct protein-protein interactions. For instance, DELLAs, PIFs, ARF6, and PKL, all directly interact with BZR1/BES1, forming a BZR1/BES1-centered regulatory network to coordinate cell elongation. By dissecting various BZR1/BES1-mediated BR responses, the concept that BZR1/BES1 act as an integration hub in multisignal-regulated plant growth and development was developed. The regulation of BZR1/BES1 is dynamic and multifaceted, including phosphorylation status, activity, and stability. Moreover, certain epigenetic modification mechanisms are involved in BZR1/BES1's regulation of gene expression. Herein, we review recent advances in BZR1/BES1-mediated molecular connections between BR and other pathways, highlighting the central role of the BZR1/BES1 interactome in optimizing plant growth and development.

1. Introduction

Plant hormones are central for the regulation of plant growth and development because they not only orchestrate intrinsic developmental programs, but also convey environmental inputs [1,2]. The plant hormone family includes, but is not limited to, auxin, gibberellin (GA), abscisic acid (ABA), cytokinin (CK), ethylene, brassinosteroid (BR), jasmonate (JA), salicylic acid (SA), and strigolactone [3]. Among them, BRs, a group of plant-specific polyhydroxylated steroidal hormones, have been extensively studied [3,4]. As a major growth-promoting hormone, BR regulates a wide range of growth and developmental events, including cell elongation, seed germination, stomata formation, vascular differentiation, plant architecture, flowering, stress resistance, male fertility, and senescence [5–9]. In particular, plants with defects in BR biosynthesis or signaling show a typical dwarf phenotype, suggesting that BR plays essential roles in normal plant growth and development [10].

Over the past decade, by combining extensive research approaches, including molecular genetics, biochemistry, proteomics, and genomics, the BR signaling pathway has become one of the best-understood signal transduction pathways in plants. A series of important BR signaling components have been identified, and thus a major BR signaling cascade has been assembled, from the cell-surface BR perception to the downstream signal transduction and subsequent transcriptional network (Fig. 1) [5,11]. In brief, the extracellular LRR domains of the BRASSINOSTEROID-INSENSITIVE 1 (BRI1) receptor and SOMATIC EMBRYOGENESIS RECEPTOR KINASE (SERK) family proteins, including BR co-receptor kinase BRI1-ASSOCIATED RECEPTOR KINASE 1 (BAK1)/SERK3, extremely twist and form an integral BR-binding pocket that percepts BR and then triggers the conformational change of the cytoplasmic domain of BRI1 and SERKs [12–17], and in turn transphosphorylates BRI1 to further enhance BRI1 kinase activity and promotes its dissociation from the negative regulator, BRI1 KINASE INHIBITOR 1 (BKI1) [15,18–20]. The activated BRI1 then triggers a subsequent series of phosphorylation events, including phosphorylation and activation of two downstream kinases, BR-SIGNALING KINASE 1

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https://doi.org/10.1016/j.bbagrm.2018.04.003
Received 1 December 2017; Received in revised form 6 April 2018; Accepted 6 April 2018
Available online 17 April 2018
1874-9399/

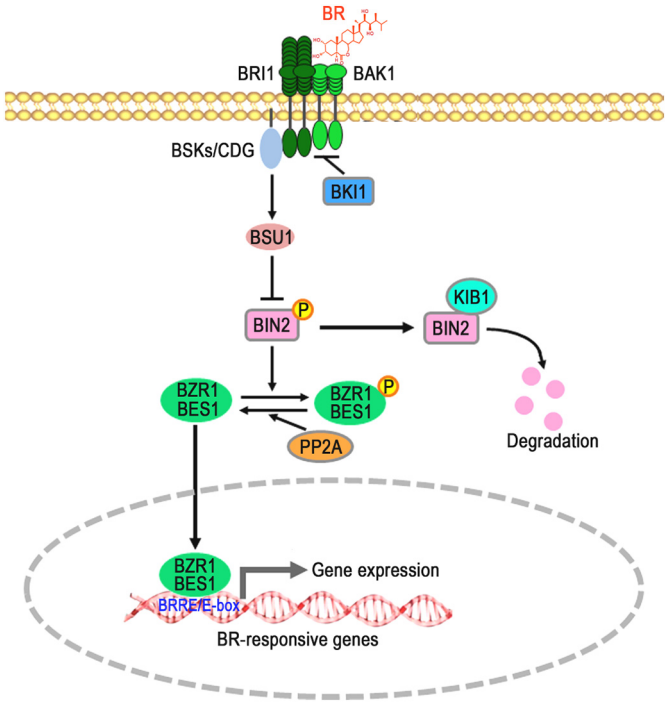

Fig. 1. The current model of the BR signaling pathway in *Arabidopsis*. BR is perceived by the extracellular domain of the BR receptor, BRI1. BR binding facilitates its heterodimerization with the co-receptor kinase BAK1, which then releases it from the inhibitory protein BKI1. Thus, the activated BRI1 sequentially phosphorylates and activates two downstream kinases, BSK1 and CDG1, and a Ser/Thr phosphatase, BSU1, which inactivates BIN2 kinase. BIN2 is the negative regulator of the BR signaling pathway, which acts by phosphorylating and inactivating the transcription factors BZR1 and BES1. The inactive BIN2 is subsequently restrained by KIB1 and facilitates its ubiquitination and degradation. BR signaling also activates phosphatase PP2A to dephosphorylate BZR1 and BES1. The dephosphorylated BZR1 and BES1 translocate into the nucleus and bind to either BRRE motif or E-box in their target genes to regulate their expression.

(BSK1) and CONSTITUTIVE DIFFERENTIAL GROWTH 1(CDG1), which successively activate BRI1-SUPPRESSOR 1 (BSU1) family phosphatases [21,22]. In turn, the activated BSU1 dephosphorylates and inactivates the GLYCOGEN SYNTHASE KINASE 3 (GSK3)-like kinase BRASSINOSTEROID-INSENSITIVE 2 (BIN2), which is the master negative regulator of the BR signaling pathway [23], to prevent it from phosphorylating and inactivating BRASSINAZOLE-RESISTANT 1 (BZR1) and BRI1-EMS-SUPPRESSOR 1 (BES1), two key transcription factors that positively mediate BR responses [14,23,24]. In the presence of BR, BIN2 is dephosphorylated by BSU1 and subsequently restrained by KINK SUPPRESSED IN BZR1-1D (KIB1), an F-box E3 ubiquitin ligase that excludes BIN2 association with its substrates BZR1/BES1, and facilitates its ubiquitination and degradation [25]. Meanwhile, BZR1 and BES1 are dephosphorylated and activated by PROTEIN PHOSPHATASE 2A (PP2A) [26]. The dephosphorylated BZR1 and BES1 then translocate from the cytoplasm to the nucleus to regulate target gene expression by directly binding to their BR-response element (BRRE, CGTGC/TG) and E-box (CANNTG) motifs, thus initiating a series of BR-responsive cellular activities and biological events [27,28].

2. BZR1 and BES1 directly regulate a large number of target genes

BZR1 represents a class of plant-specific transcription factors with five more members in *Arabidopsis*, among which BES1 is the closest homolog of BZR1, with 88% overall amino acid sequence identity [29]. BZR1 and BES1 were demonstrated as the master transcription factors in the BR signaling pathway that directly regulates the expression of many target genes [30–32]. In this review, because of the high similarity between BZR1 and BES1, we will employ a common practice in the field by using BZR1/BES1 to represent this family of transcription factors. As the essential transcription factors, BZR1/BES1 have a relatively large number of direct target genes in the *Arabidopsis* genome, including 3410 high-confidence BZR1 target genes and 1609 putative BES1 target genes, which were identified by chromatin immunoprecipitation-microarray (ChIP-chip) combined with expression profiling [30,31]. Functional classification analysis revealed that BZR1/BES1 mediate numerous molecular links between BR and other pathways. For instance, BZR1/BES1 bind to the promoters of a large number of genes involved in protein metabolism, cellular transport, cell wall biosynthesis, cell signaling, cytoskeleton, and chromatin assembling. Direct interaction between a protein and DNA represents a typical way to mediate the crosstalk between different pathways. Thus, BR regulates a wide spectrum of cellular activities and biological processes, which depend, at least in part, on the direct regulation of gene expression via the BZR1/BES1 family of transcription factors, thus eliciting various BR responses.

3. The regulation of BZR1 and BES1

3.1. BR mediated regulation of BZR1/BES1

The important roles of BZR1/BES1 in BR signaling pathway mean that the dynamic regulation of BZR1/BES1 activity and stability in response to internal and external stimuli is critical to maintain normal plant growth and development [33]. In fact, BZR1/BES1 are tightly regulated by upstream BR signaling via the sensitive modulation of its phosphorylation status. In the presence of BR, BZR1/BES1 are rapidly dephosphorylated by phosphatase PP2A. For example, PP2A directly interacts with BZR1's Pro-, Glu-, Ser-, and Thr-rich (PEST) domain, and the *bzr1-1D* mutation (P234L) enhances their interaction [26]. In the absence of BR, BZR1/BES1 are directly phosphorylated by BIN2, one of the ten GSK3-like kinases in *Arabidopsis*. BIN2 is the principal negative regulator of BR signaling and its dominant gain-of-function mutant *bin2* exhibits a dwarf phenotype, similar to BR deficient mutants [34–36]. There are about 25 putative GSK3 phosphorylation sites in BZR1/BES1; therefore, phosphorylated BZR1/BES1 exhibit an apparent mobility shift compared with dephosphorylated BZR1/BES1 [37,38]. The phosphorylation status of BZR1/BES1 has multiple effects on their stability and activity. For instance, phosphorylated BZR1/BES1 are unstable and likely to be degraded by the proteasome [39]. In addition, phosphorylated BZR1/BES1 have an altered subcellular localization and is retained in the cytoplasm [40]. Finally, the DNA-binding activity of phosphorylated BZR1/BES1 is reduced, thus attenuating their transcriptional regulation of BR-responsive target genes [40,41]. This evidence suggests that phosphorylated BZR1/BES1 represent an inactive form of BZR1/BES1. Therefore, monitoring the phosphorylation status of BZR1/BES1 is one of the most reliable biochemical indicators to study the effects of BR application.

3.2. Other components mediated regulation of BZR1/BES1

In addition to direct modulation from the two key elements of BR signaling pathway, BIN2 and PP2A, increasing evidence indicates that other effectors, independent of the BR pathway, are also involved in the regulation of BZR1/BES1 stability and activity. We will briefly summarize the recent progress in this respect. 14-3-3 proteins, phosphopeptide-binding proteins that are highly conserved in all eukaryotes, were reported to directly bind to a conserved 14-3-3 binding domain of BZR1 (amino acids RISNSCP) in both *Arabidopsis* and rice [38,40]. Phosphorylation of the second serine (S) in the 14-3-3 binding domain (RISNSCP) was proven to be essential to guarantee the interaction between BZR1 and 14-3-3. The substitution of this S with alanine (A) affects phosphorylation of this position and thereby disrupts their