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The Brassinosteroid-Activated BRI1 Receptor Kinase Is Switched off by Dephosphorylation Mediated by Cytoplasm-Localized PP2A B′ Subunits

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ABSTRACT

Brassinosteroid (BR) binding activates the receptor kinase BRI1 by inducing heterodimerization with its co-receptor kinase BAK1; however, the mechanisms that reversibly inactivate BRI1 remain unclear. Here we show that cytoplasm-localized protein phosphatase 2A (PP2A) B′ regulatory subunits interact with BRI1 to mediate its dephosphorylation and inactivation. Loss-of-function and overexpression experiments showed that a group of PP2A B′ regulatory subunits, represented by B′η, negatively regulate BR signaling by decreasing BRI1 phosphorylation. BR increases the expression levels of these B′ subunits, and B′η interacts preferentially with phosphorylated BRI1, suggesting that the dynamics of BR signaling are modulated by the PP2A-mediated feedback inactivation of BRI1. Compared with PP2A B′α and B′β, which promote BR responses by dephosphorylating the downstream transcription factor BZR1, the BRI1-inactivating B′ subunits showed similar binding to BRI1 and BZR1 but distinct subcellular localization. Alteration of the nuclear/cytoplasmic localization of the B′ subunits revealed that cytoplasmic PP2A dephosphorylates BRI1 and inhibits the BR response, whereas nuclear PP2A dephosphorylates BZR1 and activates the BR response. Our findings not only identify the PP2A regulatory B subunits that mediate the binding and dephosphorylation of BRI1, but also demonstrate that the subcellular localization of PP2A specifies its substrate selection and distinct effects on BR signaling.

Key words: Brassinosteroid, signal transduction, receptor inactivation, BRI1 dephophorylation, PP2A

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INTRODUCTION

Receptor kinases are cellular switches of signal transduction pathways regulated by extracellular signals. *Arabidopsis* possesses more than 600 receptor-like kinases, among which brassinosteroid-insensitive 1 (BRI1), the receptor of brassinosteroid (BR), is the best characterized and thus serves as a paradigm for understanding the principal mechanisms of receptor kinase activation and inactivation. While many details have been elucidated about how ligand binding activates BRI1, as well as how the BR signal is transduced to regulate gene expression, little is known about how BRI1 is inactivated when the BR level drops.

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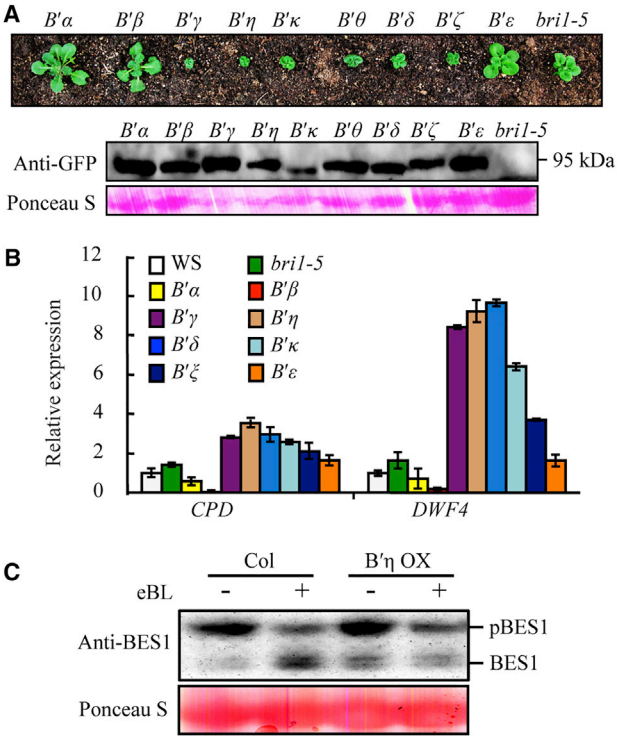

Figure 1. PP2A B′ Subunits Play Dual Roles in Regulating BR Signaling.

(A) Overexpressing different PP2A B′ subunits either suppressed (*B′α* and *B′β*) or enhanced the phenotype of the *bri1-5* mutant. The upper panel shows 3-week-old plants overexpressing the indicated B′ subunits as YFP fusions; the lower panel shows anti-GFP immunoblots of YFP-B′ expressed in the transgenic plants shown in the upper panel. Ponceau S-stained Rubisco large subunit is shown as a loading reference.

(B) qRT-PCR analysis of the *CPD* and *DWF4* expression levels in the seedlings shown in **(A)**. Error bars represent SD. Wassilewskija (WS) wild type plants were used as control.

(C) The overexpression of *B′η* inhibited eBL-induced BES1 dephosphorylation. Wild-type (Col) and *B′η*-overexpressing Col seedlings were grown side by side on $^1/_2$ MS medium for 1 week. The seedlings were then treated with 1 μM eBL for 5 min and harvested for immunoblotting using anti-BES1 antibodies (upper panel). The lower panel shows Ponceau S-stained Rubisco large subunit as a loading control.

BR signaling is initiated when BR binds to the ectodomain of BRI1 to create, together with the folding of a loop region, an interaction surface for the co-receptor kinase BRI1-associated receptor kinase 1 (BAK1) and its homolog SERK1, leading to their heterodimerization (Wang et al., 2001; Li et al., 2002; Nam and Li, 2002; Santiago et al., 2013; Sun et al., 2013). Transphosphorylation between BRI1 and BAK1 activates BRI1 and causes the dissociation of BRI1 kinase inhibitor (BKI1) (Wang and Chory, 2006), increasing the affinity between the kinase domains of BRI1 and BAK1 and further stabilizing the receptor/co-receptor complex (Li et al., 2002; Nam and Li, 2002; Wang et al., 2008). BR-activated BRI1 phosphorylates the membrane-localized receptor-like cytoplasmic kinases BSKs and CDG1, which, in turn, activate BRI1-suppressor 1 (BSU1) family phosphatases (Tang et al., 2008; Kim et al., 2011). BSU1 dephosphorylates and inactivates the GSK3/SHAGGY-like kinase brassinosteroid-insensitive 2 (BIN2) to prevent it from phosphorylating brassinazole-resistant 1 (BZR1) family transcription factors (Kim et al., 2009). Upon the BR-induced inactivation of BIN2, BZR1 is dephosphorylated by protein phosphatase 2A (PP2A) (Tang et al., 2011) and accumulates in the nucleus, where it binds to DNA to regulate the expression of BR-responsive genes (He et al., 2005; Sun et al., 2010). When BR levels are low, BZR1 is phosphorylated by BIN2 and consequently loses its DNA-binding activity; it is then retained in the cytoplasm by 14-3-3 proteins (Gampala et al., 2007; Ryu et al., 2007).

It has been shown that BRI1 is negatively regulated by several mechanisms. First, BRI1 activity is inhibited by the unphosphorylated form of its C terminus and by association with BKI1 (Wang et al., 2005; Wang and Chory, 2006). Both mechanisms appear to help maintain a low level of BRI1 activity before BR activation, rather than the inactivation of BR-activated receptor molecules, because these inhibitory effects are released by BR-induced phosphorylation. A recent genetic study suggested that the abundance of BRI1 in the plasma membrane is regulated by PP2A. BR induces the expression of a leucine carboxyl methyltransferase known as SBI1, which methylates the PP2A C subunits and causes PP2A association with the plasma membrane, possibly leading to BRI1 dephosphorylation and degradation (Wu et al., 2011). However, there is no direct evidence showing the interaction of BRI1 with PP2A or the dephosphorylation of BRI1 by PP2A in *Arabidopsis*. Furthermore, how the positive and negative effects of PP2A on BR signaling via BZR1 and BRI1, respectively, are specified and balanced remains an outstanding question.

The PP2A holoenzyme is a heterotrimeric complex composed of a catalytic C subunit, a regulatory B subunit that determines the substrate specificity, and a scaffolding A subunit that brings the B and C subunits together (Janssens and Goris, 2001). In *Arabidopsis*, the B-subunit proteins can be further grouped into three subfamilies: B, B′, and B″ (Farkas et al., 2007). Previously, we demonstrated that B′α and B′β subunits interact directly with BZR1 and target BZR1 for dephosphorylation, thereby positively regulating BR signaling (Tang et al., 2011). In this study, we provide both genetic and biochemical evidence to show that the PP2A B′ subfamily members B′γ, B′η, B′θ, B′ζ, B′ δ, and B′κ inhibit BR signaling, at least for B′η and B′γ, through a direct interaction with and the dephosphorylation of activated BRI1. Our study not only identifies the B′ subunits that mediate the dephosphorylation of BRI1 by PP2A in *Arabidopsis*, it also demonstrates that the positive (through BZR1) and negative (through BRI1) effects of PP2A on BR signaling are determined by its subcellular localization.

RESULTS

BR Signaling Is Negatively Regulated by a Set of PP2A B′ Subunits

It was reported previously that PP2A B′α and B′β positively regulate BR signaling by targeting BZR1 for dephosphorylation (Tang et al., 2011). There are nine genes encoding B′-subfamily members in the *Arabidopsis* genome (Supplemental Figure 1). Yeast two-hybrid assays have shown that four PP2A B′ subunits interact strongly with BZR1 while three interact weakly (Tang et al., 2011). To understand the functions of these B′ subunits, we overexpressed nine B′-subfamily members in weak *bri1* mutant background. Surprisingly, the overexpression of *B′η*, *B′γ*, *B′κ*, *B′δ*, *B′ζ*, and *B′θ* enhanced the semi-dwarf phenotype