Molecular Plant
Research Article

CellPress PARTNER JOURNAL

BZR1 Interacts with HY5 to Mediate Brassinosteroid- and Light-Regulated Cotyledon Opening in *Arabidopsis* in Darkness

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http://dx.doi.org/10.1016/j.molp.2015.08.014

ABSTRACT

Light and brassinosteroid (BR) are two central stimuli that regulate plant photomorphogenesis. Although previous phenotypic and physiological studies have implied possible interactions between BR and light in regulating photomorphogenesis, the underlying molecular mechanism(s) remain largely unknown. In the present study, we identified a physical connection between the BR and light signaling pathways, which was mediated by the BR-regulated transcription factor BZR1 and light-regulated transcription factor HY5 in *Arabidopsis thaliana*. Genetic evidence showed that the gain-of-function *bzr1-1D* mutant in the BR signaling pathway and loss-of-function *hy5-215* mutant in the light signaling pathway exhibited closed cotyledons under BR-deficient and dark-grown conditions and both *bzr1-1D* and *hy5-215* mutants were able to suppress the cotyledon opening phenotype of the BR-insensitive mutants *bri1-5* and *bin2-1*. Biochemical studies demonstrated that BZR1 interacts with HY5 both *in vitro* and *in vivo* and ectopic expression of HY5 considerably reduces the accumulation of BZR1 protein. In addition, HY5 specifically interacts with the dephosphorylated form of BZR1 and attenuates BZR1's transcriptional activity in regulating its target genes related to cotyledon opening. Our study provides a molecular framework for coordination of BR and light signals in regulating cotyledon opening, an important process in photomorphogenesis in plants.

Keywords: photomorphogenesis, brassinosteroid, light, HY5, BZR1, *Arabidopsis thaliana*

Li Q.-F. and He J.-X. (2016). BZR1 Interacts with HY5 to Mediate Brassinosteroid- and Light-Regulated Cotyledon Opening in *Arabidopsis* in Darkness. Mol. Plant. **9**, 113–125.

INTRODUCTION

As sessile organisms, plants must constantly adapt their growth and development to the changing environment. Plant growth and development are orchestrated by both environmental cues and intrinsic pathways (Vert and Chory, 2011). Light, the major energy source for plant growth and development, is one of the most important environmental factors affecting plant development. In *Arabidopsis thaliana*, seedling growth in bright light is characterized by shortened hypocotyls, expanded cotyledons, and self-regulating stem cell populations at root and shoot apices, a process referred to as photomorphogenesis. In contrast, seedling growth in the dark is termed skotomorphogenesis, which is typically characterized by an etiolated phenotype including elongated hypocotyls, tightly closed apical hooks, and unopened cotyledons.

Light signals are perceived by at least four families of photoreceptors, including phytochromes, cryptochromes, phototropins, and UV Resistance Locus 8 (UVR8) (Casal, 2013). Downstream of these photoreceptors are several classes of transcription factors, including the bHLH proteins Phytochrome-Interacting Factors (PIFs) (Leivar and Quail, 2011) and the bZIP protein Elongated Hypocotyl 5 (HY5) (Osterlund et al., 2000). Most of the transcription factors directly bind to the G-box of their target genes and therefore modulate their expression in response to light (Jiao et al., 2007). In addition to the receptors and transcription factors, another group of proteins termed constitutive photomorphogenic/de-etiolated/fusca (COP/DET/FUS), which are central repressors of

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photomorphogenesis, also play important roles in the light signaling pathway. These proteins belong to the light-regulated ubiquitination system or COP9 signalosome and mediate the degradation of the transcription factors (Wei and Deng, 1996; Lau and Deng, 2012; Huang et al., 2014).

Brassinosteroids (BRs), a group of steroidal hormones in plants, are important endogenous signals for the regulation of plant growth and development. BR and light affect many common developmental and physiological processes such as seed germination, flowering, and hypocotyl elongation (Kim and Wang, 2010). Like light, BR also plays important roles in plant photomorphogenesis. Dark-grown BR-deficient and -insensitive mutants exhibit de-etiolation phenotypes, including shortened hypocotyls and opened cotyledons (Chory et al., 1991; Li et al., 1996; Wang et al., 2012), suggesting that BRs are also involved in the regulation of plant photomorphogenesis, but little is known on how BR signals coordinate with light signals to regulate plant photomorphogenesis. The recent advances in understanding of BR signal transduction mechanisms have made it possible to address this question. In *Arabidopsis*, BR signals are perceived by the transmembrane leucine-rich-repeat containing receptor-like kinase (LRR-RLK) BRI1 (Li and Chory, 1997) and BR binding results in fast activation of BRI1's intracellular kinase domain. The activated BRI1 triggers a downstream phospho-relay signal transduction cascade and culminates in dephosphorylation and nuclear localization of two closely related transcription factors BZR1 and BES1, which bind the 5′-CGTG(T/C)G-3′ elements (BRRE) and 5′-CANNTG-3′ E-box of their target genes (He et al., 2005; Yin et al., 2005; Sun et al., 2010; Yu et al., 2011). BIN2, a GSK3/Shaggy-like protein kinase, functions as a negative regulator of BR signaling by phosphorylating and inactivating BZR1 and BES1 (He et al., 2002; Li and Nam, 2002; Yin et al., 2002; Zhao et al., 2002).

Study of the crosstalk between light and BR signaling pathways has been an attractive subject for plant biologists as these factors are the key environmental stimulus and endogenous signals for plant growth and development, respectively. Although early phenotypic characterizations of BR-deficient and -insensitive mutants had indicated crosstalk between light and BR signaling in regulating photomorphogenesis (Li et al., 1996; Szekeres et al., 1996; Chory and Li, 1997; Azpiroz et al., 1998), the underlying molecular mechanisms have only begun to be understood recently. For example, a recent study by Luo et al. (2010) showed that the transcription factor BZR1 in the BR pathway was able to interact with GATA2, a transcription factor in the light signaling pathway, to regulate hypocotyl elongation of seedlings. BZR1 directly bound the promoter of *GATA2* and repressed its transcription, thereby inhibiting plant photomorphogenesis. BZR1 was also found to interact with Phytochrome-Interacting Factor 4 (PIF4), another key transcription factor in light signaling, to coordinate BR- and light-regulated cell elongation processes by co-regulating a large number of common target genes, including those required for cell elongation (Oh et al., 2012). More recently, direct interaction between BZR1 and COP1, a dark-activated ubiquitin E3 ligase, was also reported. COP1 was able to capture and degrade the inactive form of BZR1 and thus increase the ratio of the active form of BZR1, thereby promoting hypocotyl growth through enhanced BR signaling (Kim et al., 2014). These studies suggested a complex regulation network that integrates the BR and light signals in programming plant growth and development. However, these studies were more concerned with the hypocotyl elongation phenotypes of plant seedlings; how BR and light coordinate to regulate other photomorphogenic processes is unclear. For instance, both light and BR are involved in the regulation of cotyledon opening and apical hook formation in *Arabidopsis*, which are crucial for plants to protect their shoot apical meristems from damage when germinated seedlings grow toward the soil surface (Arsovski et al., 2012). Indeed, hookless mutants are not able to emerge when seeds are buried in the soil and germinated (Harpham et al., 1991). How BR and light signals coordinate to regulate these processes is not yet known.

In this study, we found that the *hy5-215* mutant that is defective in light-regulated hypocotyl elongation also has closed cotyledons under BR-deficient conditions, which resembles *bzr1-1D*, a gain-of-function mutant of BZR1 in BR signaling. Further studies indicated that HY5 can modulate the BR signaling pathway to regulate cotyledon opening and light can affect the phosphorylation status and abundance of the BZR1 protein. More interestingly, BZR1 interacts with HY5 *in vitro* and *in vivo* and overexpression of HY5 diminishes the *in vivo* accumulation of BZR1. HY5 was also found to specifically bind the dephosphorylated form of BZR1 (the active form) and antagonize BZR1's transcriptional activity in regulating cotyledon development and opening related genes.

RESULTS

Both *bzr1-1D* and *hy5-215* Mutants Have Closed Cotyledons under BR-Deficient Conditions

Light is the primary regulator of plant photomorphogenesis. *Arabidopsis* seedlings grown in darkness exhibit etiolated phenotypes, characterized by elongated hypocotyls and tightly closed cotyledons. In contrast, seedlings grown in the light have short hypocotyls, expanded and photosynthetically active cotyledons. Besides light, the plant hormone BR is also involved in this process. When grown in the dark, the BR-deficient mutant *det2* and BR-insensitive mutants *bri1-5* and *bin2-1* all show de-etiolated phenotypes, including short hypocotyls and opened cotyledons (Figure 1A). *bzr1-1D*, a dominant mutant of BZR1, shows a similar phenotype to its wild-type Col-0 under normal dark-growth conditions (Figure 1A). However, when treated with brassinazol (BRZ), a BR biosynthesis inhibitor, the cotyledons of Col-0 plants became opened, whereas those of *bzr1-1D* remained closed (Figure 1B and Supplemental Figure 1A). Consistent with previous findings that the constitutive photomorphogenic phenotype of BR-insensitive mutants can be suppressed by *bzr1-1D* (Wang et al., 2002), our crossing results also demonstrated that the opened apical hook phenotype of the *bri1-5* mutant can be suppressed by *bzr1-1D* (Figure 1C), which suggests that BR inhibits cotyledon opening in the dark via the transcription factor BZR1. Since light and BR antagonistically regulate photomorphogenesis, we next examined the response of several light-related mutants (including *phA-211*, *phyB-9*, *phyA-211 phyB-9* double mutant and *hy5-215*) to BRZ treatment in the dark to determine which genes in the light