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Integration of Light- and Brassinosteroid-Signaling Pathways by a GATA Transcription Factor in *Arabidopsis*

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SUMMARY

Light and brassinosteroid (BR) antagonistically regulate the developmental switch from etiolation in the dark to photomorphogenesis in the light in plants. Here, we identify GATA2 as a key transcriptional regulator that mediates the crosstalk between BR- and light-signaling pathways. Overexpression of GATA2 causes constitutive photomorphogenesis in the dark, whereas suppression of GATA2 reduces photomorphogenesis caused by light, BR deficiency, or the constitutive photomorphogenesis mutant *cop1*. Genome profiling and chromatin immunoprecipitation experiments show that GATA2 directly regulates genes that respond to both light and BR. BR represses *GATA2* transcription through the BR-activated transcription factor BZR1, whereas light causes accumulation of GATA2 protein and feedback inhibition of GATA2 transcription. Dark-induced proteasomal degradation of GATA2 is dependent on the COP1 E3 ubiquitin ligase, and COP1 can ubiquitinate GATA2 in vitro. This study illustrates a molecular framework for antagonistic regulation of gene expression and seedling photomorphogenesis by BR and light.

INTRODUCTION

Light and brassinosteroid (BR) are key signals that determine the development program of young seedlings. To reach the surface of soil, seedlings that germinate in the dark undergo skotomorphogenesis, exhibiting elongated hypocotyls, small and folded cotyledons with undifferentiated chloroplasts, and repression of light-induced genes. Exposure to light causes a developmental switch from skotomorphogenesis to photomorphogenesis, resulting in short hypocotyls, open and expanded cotyledons, and differentiation of chloroplast (Wei and Deng, 1996). Genetic studies have identified many components that mediate this developmental switch by light. Two classes of photoreceptors, phytochrome and cryptochrome, perceiving red/far-red and blue light, respectively, play major roles in promoting photomorphogenesis. A group of proteins termed constitutive photomorphogenic/de-etiolated/fusca (COP/DET/FUS), which are components of the ubiquitination system or COP9 signalosome, are central repressors of photomorphogenesis (Deng et al., 1991; Wei and Deng, 1996). Several classes of transcription factors, such as the b-ZIP protein long hypocotyl 5 (HY5) and the phytochrome interacting factor (PIF) family of b-HLH proteins, directly regulate light-responsive gene expression and are degraded by the ubiquitin system in a light-dependent manner (Chen et al., 2004; Leivar et al., 2008; Ma et al., 2002; von Arnim et al., 1997; Wang et al., 2001). Through these components, light turns on a transcriptional program that supports photomorphogenic development (Chen et al., 2004; Jiao et al., 2007).

In addition to these light-signaling components, BR also plays a key role in photomorphogenesis. BR-deficient mutants show typical de-etiolation phenotypes in the dark, with elevated expression of many light-induced genes (Chory et al., 1991; Li et al., 1996; Song et al., 2009; Szekeres et al., 1996). Although light inhibits hypocotyl elongation and promotes chlorophyll accumulation, BR promotes hypocotyl elongation and reduces chlorophyll level. BR is perceived by the cell surface receptor kinase BRI1, and downstream signal transduction activates the BZR family transcription factors (Gendron and Wang, 2007), which mediate BR-responsive gene expression (He et al., 2005). Recent studies have established a complete BR-signal transduction pathway from the BRI1 to the BZR transcription factors (Kim et al., 2009; Tang et al., 2010; Kim and Wang, 2010). Activation of BZR1 and BZR2 is essential for skotomorphogenesis because the constitutive photomorphogenesis phenotype of BR-deficient or insensitive mutants are suppressed by the dominant *bzr1-1D* and *bes1-D* mutations, which cause constitutive activation of BR-responsive gene expression (Wang et al.,

2002; Yin et al., 2002). It has been proposed that light might inhibit BR synthesis or signaling to inhibit skotomorphogenesis and promote photomorphogenesis (Kang et al., 2001). However, no significant difference in BR level was observed between dark-grown and light-grown plants (Symons et al., 2008). On the other hand, physiological studies of BR-deficient *Arabidopsis* suggested that BR regulates phytochrome- and cryptochrome-mediated responses (Luccioni et al., 2002; Neff et al., 1999). The molecular mechanism of such BR-light interactions has remained unclear.

Analyses of light-responsive promoters have identified a number of light-response promoter elements (LREs), including the G-box, GATA, and GT1 motifs (Terzaghi and Cashmore, 1995). It has been suggested that combinations of LREs, rather than individual elements, confer proper light responsiveness to a promoter (Puente et al., 1996; Terzaghi and Cashmore, 1995). For example the combination of G-box with GATA element is critical for promoter activation in response to the signals from multiple photoreceptors as well as for repression by the COP/DET system (Chattopadhyay et al., 1998b). Most of the light-signaling transcription factors identified so far bind to the G-box (Liu et al., 2008; Jiao et al., 2007). The transcription factor that regulates light-responsive genes through the essential GATA element has not been identified in plants (Arguello-Astorga and Herrera-Estrella, 1998; Chattopadhyay et al., 1998b; Jiao et al., 2007; Terzaghi and Cashmore, 1995). In fungi, such as *Neurospora*, two GATA-type factors bind to GATA element and regulate gene expression in response to light signal (Scazzocchio, 2000). It has long been proposed that members of the *Arabidopsis* GATA family of transcription factors might play a similar role (Jeong and Shih, 2003; Manfield et al., 2007); however, genetic evidence for this hypothesis is absent.

In this study we identify a GATA-type transcription factor (GATA2) as a junction between light and BR pathways. Overexpression and loss-of-function experiments demonstrate that GATA2 is a major positive regulator of photomorphogenesis that mediates a gene expression profile with significant overlap to those caused by light treatment or BR deficiency. BR-activated BZR1 directly represses GATA2 transcription, whereas light signaling stabilizes the GATA2 protein, likely by inhibiting a COP1-dependent degradation process. The results demonstrate that GATA2 is not only a key light-signaling transcription factor but also a junction for the crosstalk between the BR- and light-signaling pathways. The results support a mode of BR-light antagonism through transcriptional and posttranslational regulation of common transcription factors.

RESULTS

GATA2 Is a Positive Regulator of Photomorphogenesis

The suppression of the photomorphogenesis phenotype of *bri1* by the *bzr1-1D* mutation suggests that BR inhibits photomorphogenesis through BZR1 and its downstream target genes. Based on BR-responsive expression and the presence of BR-response elements in their promoters (He et al., 2005), two BR-repressed genes encoding GATA-type transcription factors, *GATA2* and *GATA4*, were considered putative target genes of BZR1. Because previous studies of GATA sequence in light-responsive promoter implicated unknown GATA factors in light-responsive gene expression (Chattopadhyay et al., 1998b), we tested whether GATA2 and GATA4 play a role in light- or BR-regulated gene expression and photomorphogenesis.

GATA2 and GATA4 are the two closest members of the subfamily I of *Arabidopsis* GATA factors (Reyes et al., 2004). Quantitative RT-PCR analysis confirmed that the transcript level of *GATA2* is reduced by BR treatment. GATA2 is expressed at a higher level in the dark than in the light, and BR repression is also more obvious in the dark than in the light (Figure 1A). *GATA2* RNA level is increased in the BR-deficient mutant *det2* and BR-insensitive mutant *bri1-116* but repressed by the *bzr1-1D* mutation (Figure 1B). A *GATA2* promoter-GUS reporter gene showed strong expression in hypocotyls and petioles (see Figure S1 available online), where cell elongation is most sensitive to light and BR. *GATA2* expression was also detected in root tips, the junctions of floral organs, and styles of plants grown under light (Figure S1). RT-PCR assays confirmed ubiquitous expression of *GATA2* in various tissues (Figure S1I). A GATA2-YFP fusion protein is localized in the nucleus (Figures S1J–S1O). Such expression pattern and subcellular localization of GATA2 are consistent with a role as transcription factor for photomorphogenesis. Recent coexpression analysis has shown that *GATA2* and *GATA4* show strong coexpression with each other (Manfield et al., 2007).

To investigate the function of these GATA factors, we generated transgenic plants overexpressing *GATA2* and *GATA4* under the control of the cauliflower mosaic virus 35S promoter (*GATA-ox*). Of five *GATA2-ox* transgenic lines, four lines exhibited obvious short hypocotyls and open cotyledons in the dark, similar to the BR-deficient or insensitive mutants (Figures 1C and 1D; Figures S1P and S1Q). Similarly, five of ten *GATA4-ox* lines also showed shorter hypocotyl phenotypes; however, the overall phenotypes were weaker than the *GATA2-ox* lines (Figure S1R). We further generated *GATA2* antisense (*GATA-AS*) and artificial microRNA (*GATA-AM*) transgenic plants. The constructs contain conserved sequence and are expected to also suppress *GATA4*. Many *GATA-AS* and *GATA-AM* lines showed long hypocotyl phenotypes in the light (Figure 1E; Figure S2A), but not in the dark (Figure S2G). These results demonstrate that GATA2 plays an important role in promoting photomorphogenesis, and GATA4 is likely to play a similar but less prominent role.

As positive regulator of photomorphogenesis, the increased expression of GATA2 in BR mutants is likely to contribute to the de-etiolation phenotypes. To determine if GATA2 plays a role in BR regulation of photomorphogenesis, we crossed the *GATA-AS* and *GATA-AM* lines with the BR-deficient mutant *det2* and BR-insensitive mutant *bin2*, and these plants exhibited longer hypocotyls than the *det2* and *bin2* single mutants (Figures 2A and 2B; Figures S2B and S2C). We also crossed the *GATA2-ox* line with *bzr1-1D*, which suppresses the de-etiolation phenotypes of the BR-biosynthetic or signaling mutants (Figure 1D). Seedlings homozygous for both *GATA2-ox* and *bzr1-1D* had short hypocotyls and open cotyledon in the dark, resembling the phenotype of *GATA2-ox* (Figure 2C; Figure S2D), consistent with GATA2 acting downstream of BZR1. These results support an important role of repressing GATA2 in BR inhibition of photomorphogenic development.

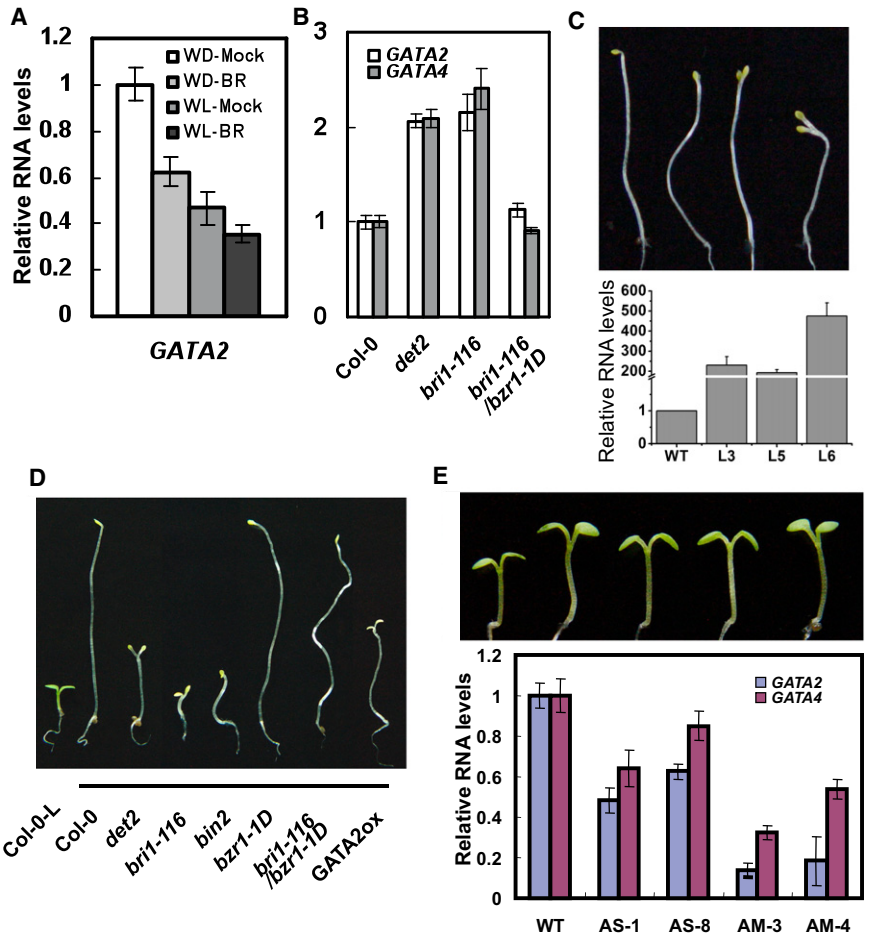

Figure 1. GATA2 Is a Positive Regulator of Photomorphogenesis

(A) BR treatment reduces *GATA2* RNA level. *Arabidopsis* seedlings grown in the dark (WD) or light (WL) for 5 days were treated with mock solution or 100 nM 24-epibrassinolide (BR) for 3 hr, and the expression of *GATA2* was analyzed by qRT-PCR.

(B) qRT-PCR analysis of *GATA2* and *GATA4* RNA levels in 5-day-old dark-grown WT (Col-0), *det2*, *bri1-116*, and *bri1-116 bzr1-1D*.

(C) Dark-grown phenotypes of three *GATA-ox* lines. Lower panels show qRT-PCR of *GATA2* expression (see also Figures S1P and S1R).

(D) Phenotypes of light-grown (first on left) or dark-grown seedlings of WT (Col-0), BR mutants, and a representative *GATA2-ox* transgenic line 6.

(E) Phenotypes of antisense (AS) or artificial-microRNA (AM) transgenic *Arabidopsis* seedlings with reduced levels of *GATA2* and *GATA4* (see also Figure S2A for quantitation data). Lower panel shows qRT-PCR analysis of *GATA2* and *GATA4* in these transgenic seedlings. All error bars are standard deviation (SD).

Light regulates seedling development through several photoreceptor families that absorb light of distinct wavelengths. To test if GATA2 functions in any specific photoreceptor pathway, we grew the *GATA2-ox* and *GATA*-knockdown lines under monochromatic red, far-red, or blue light. The *GATA2-ox* plants had shorter hypocotyls, and the *GATA-AS* or *GATA-AM* plants showed longer hypocotyls under all wavelengths of light, but not in the dark (Figures 2D and 2E; Figures S2E–S2G), suggesting that GATA2 is likely to function downstream of all photoreceptors. Fluence-rate response analyses indicate that the *GATA2-ox* plants have enhanced sensitivity, and the *GATA-AS* and *AM* plants have reduced sensitivity to light (Figures 2E and 2F; Figure S2G). To test if GATA2 is downstream of the master photomorphogenic repressor COP1 (Deng et al., 1991), we crossed the *GATA-AS* and *GATA-AM* lines into the *cop1-4* and *cop1-6* mutants. Knockdown of GATA partly suppressed the de-etiolation phenotypes of the *cop1* mutants (Figure 2G; Figure S2H), suggesting that GATA2 functions downstream of COP1 in the light-signaling pathway.

GATA2 Overexpression Causes Similar Transcriptomic Changes as Light and BR Deficiency

To further understand the function of GATA2 in the light- and BR-signaling pathways, we compared the transcriptomic changes caused by *GATA2* overexpression, *bri1* mutation, and light treatment. Four-day-old dark-grown seedlings of *GATA2-ox*, *bri1-116*, and wild-type (WT) were analyzed by microarray using the ATH1 array (Affymetrix). The results showed that expression of 2910 genes was altered in *GATA2-ox* plants, with 1743 genes repressed and 1167 activated by *GATA2* overexpression (>2-fold and $p < 0.05$) (Table S1A). In the *bri1-116* mutant, 2992 genes were differentially expressed compared to WT, and about 38% (1144 of the 2992) of them were also affected in *GATA2-ox* (Figure 3A; Table S1B). More striking overlap was observed for the 120 most-repressed genes in *GATA2-ox*: 103 (86%) of them were also repressed in *bri1-116* (Table S1C). Overall, about 93% (1055) of the 1144 coregulated genes were affected in the same way by *GATA2-ox* and *bri1-116* (Figure 3B; Table S1D). Such similar genomic effects of *GATA2-ox* and *bri1* mutation are consistent with elevated *GATA2* expression in *bri1*, contributing to its altered gene expression and de-etiolation phenotype.

When the gene expression changes of *GATA2-ox* were searched against an *Arabidopsis* microarray database that includes 1450 treatments (Zhang et al., 2010), the top nine best matches were microarray experiments that compared seedlings grown under various light conditions to those grown in the dark. The percent overlaps with the light data sets ranged from 27% to 48% (Table S2). The Pearson correlation coefficients of pairwise comparison between the *GATA2-ox* versus WT data and various light versus dark data range from 0.57 to 0.75 (Table S2), suggesting that *GATA2* overexpression causes a similar genomic response as light exposure. About 47% (1378) of the genes affected in *GATA2-ox* were affected by at least one of the light conditions

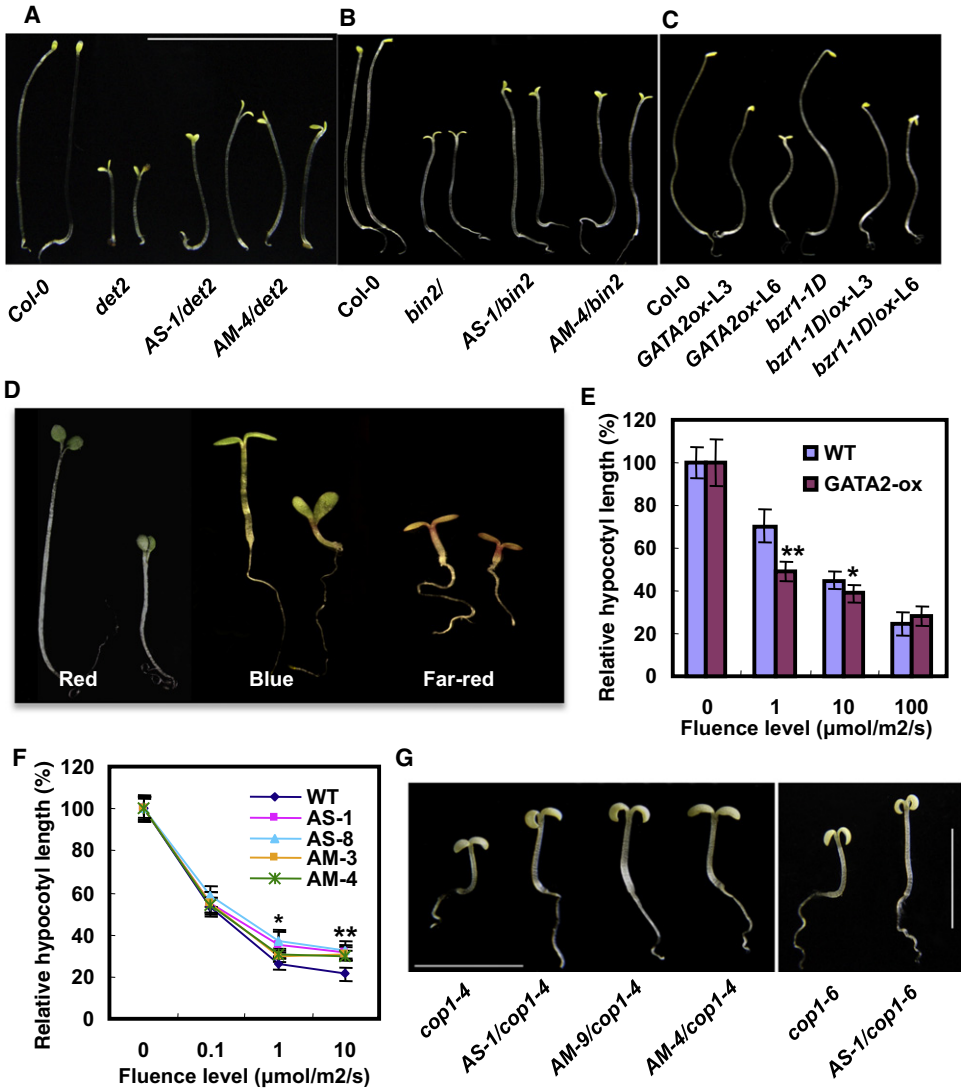

Figure 2. GATA2 Acts Downstream of Both BR- and Light-Signaling Pathways to Promote Photomorphogenesis

(A) Phenotypes of *det2* mutants crossed with the *GATA2/4* antisense (AS) or artificial microRNA (AM) lines (see also Figure S2B).

(B) Phenotypes of the *bin2* mutant crossed with the *GATA-AS* and *-AM* lines (see also Figure S2C).

(C) Phenotypes of *bzr1-1D* mutants crossed with *GATA2-ox* (see also Figure S2D).

(D) *GATA2-ox* plants (right of each pair) have short hypocotyls than WT (left) when grown under red (26 μmol/m^2/s), blue (13 μmol/m^2/s), and far-red (100 μmol/m^2/s) light conditions (see also Figure S2E).

(E) Relative hypocotyl lengths of *GATA2-ox* seedlings (L3 line) grown under various fluence rates of red light.

(F) Fluence-rate response curve of hypocotyl lengths of *GATA2-AS* and *-AM* lines grown in the dark or various intensities of blue light. Error bars in (E) and (F) are SD, and significant differences from WT are marked (**p < 0.01, *p < 0.05).

(G) Phenotypes of dark-grown *cop1* mutants crossed with *GATA-AS* or *GATA-AM* lines (see also quantitation data in Figure S2I). The seedlings were grown for 7 days.

(Table S3). Among these, 802 genes were affected by *bri1* mutation (Figure 3A; Table S1E). About 87% of these shared genes were upregulated or downregulated similarly by *GATA2-ox*, the *bri1-116* mutation, and light treatments (Figure 3B; Table S1E). Such similar effects of *GATA2* overexpression, *bri1* mutation, and light on large numbers of genes strongly support an important role for GATA2 in mediating the antagonistic effects of BR and light on gene expression and photomorphogenesis.

GATA2 Directly Regulates Genes that Respond to Light and BR Deficiency

Quantitative RT-PCR assays confirmed that the expression levels of light-repressed genes, such as *TIP2* and *IAA6*, were repressed in *GATA2-ox* and *bri1-116* plants but increased in the *GATA-AS* and *GATA-AM* plants, whereas the levels of light-induced genes, such as *CAB2*, *PSAH2*, were increased in *GATA2-ox* and *bri1-116* plants but reduced in the *GATA-AS* and *GATA-AM* plants (Figure 3C). Chromatin immunoprecipitation

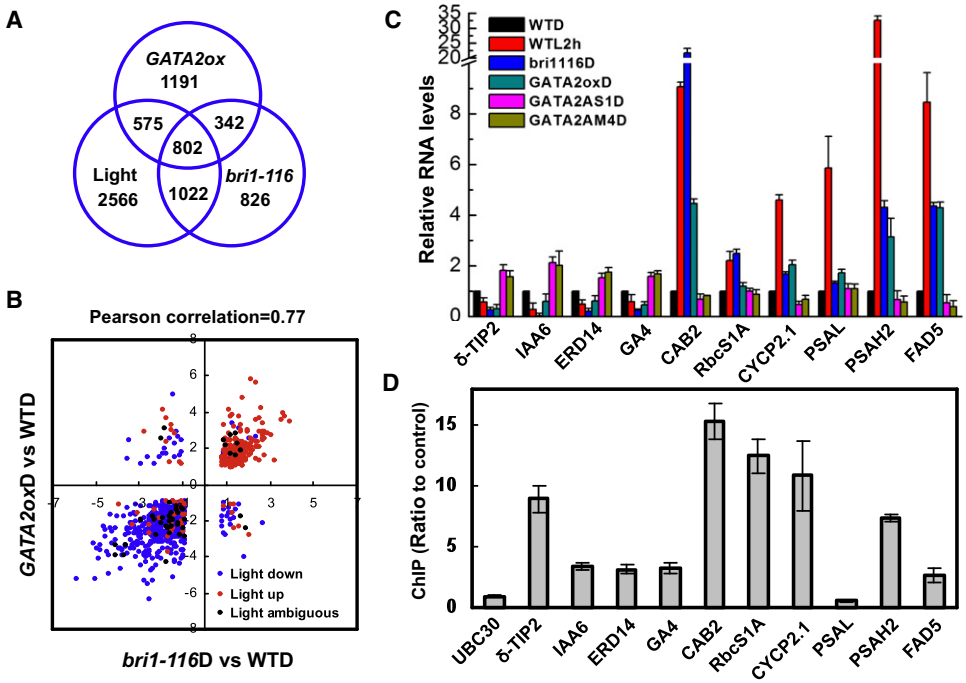

Figure 3. GATA2 Directly Regulates Genes that Are Responsive to Both BR and Light

(A) Venn diagrams of the number of genes differentially expressed in the dark-grown *GATA2-ox* versus WT, genes affected in the *bri1-116* mutant, and genes affected in at least one of the light-treatment microarray experiments (see also Table S1). The numbers in the overlapping areas indicate the number of shared genes.

(B) Scatter plot of log2 fold change values of *GATA2-ox* versus WT and *bri1-116* versus WT for 802 genes differentially expressed in dark-grown *GATA2-ox* versus WT, *bri1-116* versus WT, and light-grown versus dark-grown WT seedlings. Effects of light treatment on the expression are denoted by color as shown (see also Table S1).

(C) Quantitative RT-PCR analysis of a number of known light-responsive genes in *GATA2-ox*, *GATA-AS-1*, *GATA-AM-4*, or *bri1-116* plants grown in the dark for 5 days, compared to WT plants grown in the dark and then untreated or treated with white light for 2 hr.

(D) ChIP-qPCR assays of GATA2 binding to promoters of genes in (C), performed using *35S::GATA2-YFP* transgenic and WT control seedlings grown in light for 2 weeks and an anti-GFP antibody. GATA2 binding was measured by qPCR as the ratio between GATA2-YFP and control sample. The *UBC30* gene was used as a negative control. Error bars indicate SD (see also Table S4).

(ChIP) assays for GATA sequence-containing regions of promoters demonstrated that GATA2 bound strongly to the promoters of *TIP2*, *CAB2*, *CYCP2.1*, *RBCS1A*, and *PSAH2*, and bound weakly *to IAA6*, *ERD14*, *GA4*, *and FAD5*, which are responsive to light treatment and affected in *bri1* and the *GATA2* transgenic plants. In contrast, GATA2 did not bind to *PSAL*, which is a light-responsive gene not affected by *bri1* or *GATA-AS* (Figure 3D; Table S4). Furthermore, ChIP assays showed GATA2 binding to additional seven genes strongly repressed and three genes strongly activated in *GATA2-ox*, but not to the control gene *UBC30* or two *LHCB* genes that were not affected in *GATA2-ox* (Table S4). These results demonstrate that GATA2 directly activates some of the light-induced and BR-repressed genes and inhibits light-repressed and BR-induced genes.

Light Induces Accumulation of GATA2 Protein, which Directly Feedback-Inhibits Its Own Transcription

As a positive regulator of photomorphogenesis, *GATA2* is expected to be activated by light. However, *GATA2* and *GATA4* are expressed at a higher level in dark-grown plants than in light-grown plants (Manfield et al., 2007). Quantitative RT-PCR analysis showed that the transcript levels of *GATA2* and *GATA4* rapidly decreased upon light treatment of dark-grown seedlings (Figure 4A). Interestingly, immunoblot analysis demonstrated that light treatment increased the GATA2 protein accumulation (Figure 4B). Opposite responses at protein and RNA levels are often caused by feedback inhibition of transcription by the protein product of the gene. Indeed, the levels of the endogenous *GATA2* and *GATA4* RNAs were reduced in the *GATA2-ox* transgenic plants, which overexpress the *GATA2* RNA from the transgene (Figure 4C). Overexpression of *GATA2* also led to reduced expression of a *GATA2-GUS* reporter gene in tobacco leaf cell (Figure S3). ChIP assays further showed that the GATA2 protein directly binds to its own promoter in vivo (Figure 4D). These results demonstrate that light induces GATA2 protein accumulation at a posttranscriptional level, and light-activated GATA2 protein feedback inhibits the transcription of *GATA2* and *GATA4*.

GATA2 Is Degraded in the Dark by the Proteasome in a COP1-Dependent Manner

Several light-signaling transcription factors, such as HY5 and HFR1, are targeted for proteasomal degradation by the COP1 ubiquitin ligase, and light signaling stabilizes these transcription factors by inactivating COP1 (Osterlund et al., 2000; von Arnim

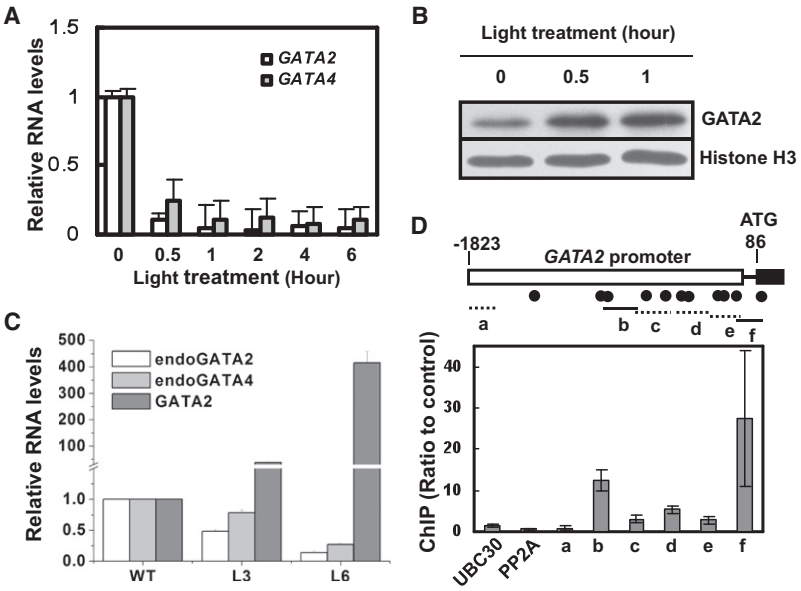

Figure 4. Light Regulates GATA2 Accumulation at the Posttranslational Level

(A) Light represses *GATA2* and *GATA4* transcription levels. Dark-grown *Arabidopsis* seedlings were treated with white light for indicated time, and RNA levels of *GATA2* and *GATA4* were measured by real-time qRT-PCR. Error bars indicate standard deviation.

(B) Light promotes GATA2 protein accumulation. Immunoblot analysis of GATA2 protein in 5-day-old dark-grown *GATA2-ox* L6 line seedlings treated with white light for the indicated time.

(C) qRT-PCR analysis of the levels of RNA expressed from the endogenous *GATA2* and *GATA4* genes (endo) or total GATA2 RNA level in WT and the *GATA2-ox* transgenic seedlings (L3 and L6). *UBC30* was used as internal control.

(D) ChIP-qPCR analysis of GATA2 binding to its own promoter. The upper panel shows a diagram of the promoter (open box), 5′ UTR (black line) and the first exon (black box) of the *GATA2* gene. Black circles indicate positions of putative GATA motifs. Lines marked a–f show GATA2-binding (solid) and nonbinding (dashed) regions analyzed by qPCR. The lower panel shows ChIP-qPCR data. Error bars indicate SD.

et al., 1997). We investigated whether a similar COP1-dependent process is involved in light regulation of GATA2 accumulation. Treatment of dark-grown seedlings with MG132, an inhibitor of the proteasome, caused GATA2 protein accumulation (Figure 5A), indicating that GATA2 is degraded by the proteasome in the dark. Upon transition from light to dark, GATA2 protein level decreased dramatically, and this decrease was blocked by MG132 treatment (Figure 5A), suggesting that light inhibits proteasomal degradation of GATA2. Immunoblotting data showed that the GATA2 protein level was increased in the *cop1* mutants grown in the dark (Figure 5B), indicating that GATA2 degradation requires COP1. The accumulation of GATA2 obviously contributes to the de-etiolation phenotype of *cop1*, because suppressing *GATA2* RNA levels in the *cop1* mutant reduced the GATA2 protein level and increased the hypocotyl length (Figures 2G and 5B). Furthermore, in vitro pull-down assays showed that COP1 can directly interact with GATA2 (Figure 5C). In vitro ubiquitination assay confirmed that COP1 can ubiquitinate GATA2 in vitro (Figure 5D). These results strongly support the possibility that the GATA2 protein is negatively regulated by COP1-dependent ubiquitination and proteasomal degradation, and inactivation of COP1 by light signaling leads to GATA2 accumulation.

BZR1 Binds to the *GATA2* Promoter In Vivo

To test if BZR1 directly regulates *GATA2* expression, we performed ChIP followed by quantitative PCR (ChIP-qPCR) assays using *pBZR:*BZR1-CFP transgenic plants and anti-GFP antibody, with 35S-GFP transgenic plants as a control. As shown in Figure 6A, BZR1 bound strongly to the *GATA2* promoter in the dark-grown seedlings but only weakly in the light-grown seedlings, consistent with a prominent role of BR in repressing *GATA2* expression in the dark (Figure 1A). In contrast, BZR1

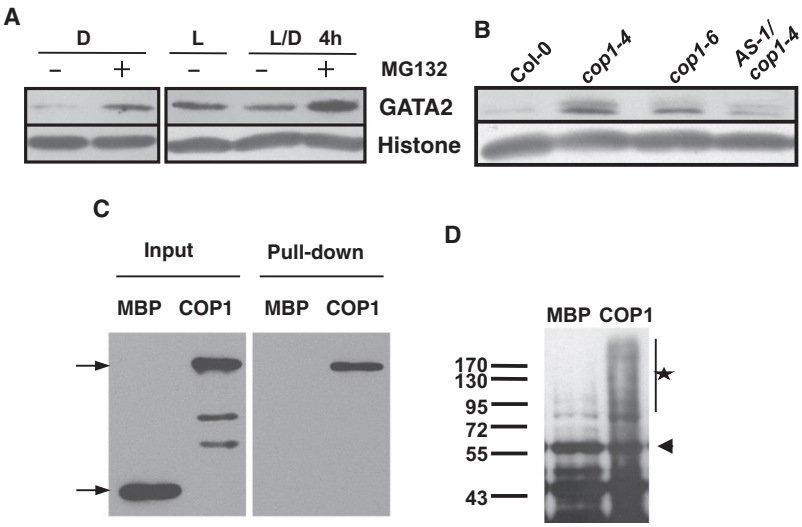

Figure 5. Light Regulates GATA2 Accumulation through a COP1 Ubiquitin Ligase-Dependent Process

(A) Immunoblot analysis of GATA2 protein levels. Dark-grown (D) or light-grown (L or L/D) 5-day-old *35S:GATA2* transgenic seedlings (L3 line) were treated with mock solution (−) or 10 μM MG132 (+) for 4 hr in the dark (D and L/D) or light (L). Histone H3 was probed as a loading control.

(B) Immunoblot assay of GATA2 protein level in 5-day-old dark-grown WT, the *cop1* mutants, and *cop1–4* crossed with the *GATA-AS* line.

(C) In vitro pull-down assay showing the interaction between GATA2 and COP1.

(D) In vitro ubiquitination assay showing ubiquitination of GST-GATA2 by MBP-COP1. The arrow points to the GST-GATA2 band, and the star marks the ubiquitinated GST-GATA2 bands.

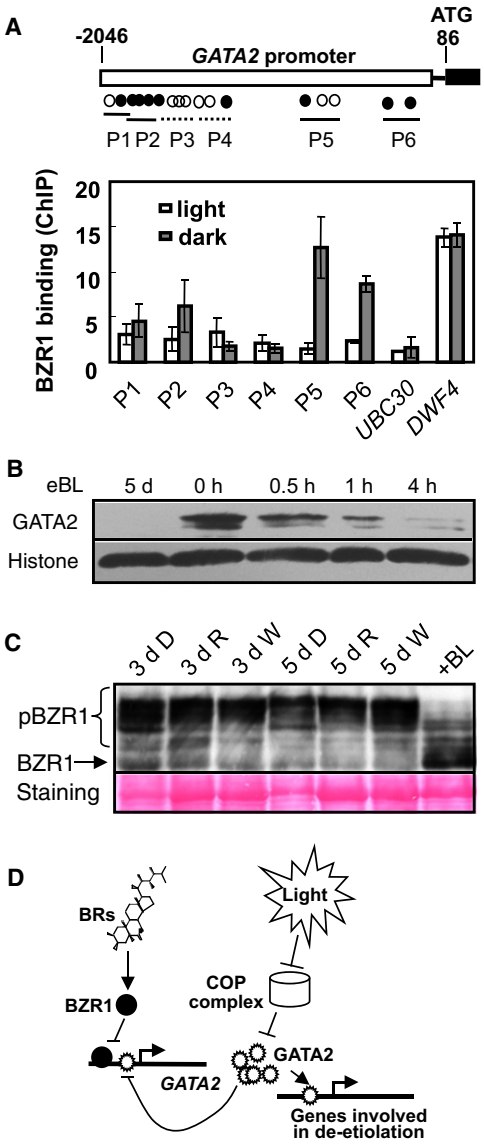

Figure 6. BR Represses *GATA2* Transcriptional Level through BZR1 Direct Binding to Its Promoter

(A) ChIP-qPCR assays of BZR1 binding to the *GATA2* promoter. The *pBZR1::BZR1-CFP* and *35S::GFP* transgenic *Arabidopsis* seedlings grown in dark or light for 5 days were used in ChIP using anti-GFP antibody. The upper panel shows a diagram depicting the putative promoter (open box), 5′ UTR (black line), and the first exon (black box) of the *GATA2* gene. Open and black circles indicate the positions of putative E-box and BRRE motifs, respectively. Thin lines marked P1–P6 show BZR1-binding (solid) and nonbinding (dashed) regions analyzed by qPCR. The lower panel shows the qPCR data for enrichment as ratio between BZR1-CFP and 35S-GFP normalized to the *CNX5* control gene. Error bars indicate SD.

(B) Immunoblot analysis shows BR repression of GATA2 accumulation. The *det2* seedlings were grown in the dark on medium with 100 nM 24-epibrassinolide (eBL) for 5 days or grown without eBL for 5 days and then treated with 10 μM eBL for 0–4 hr. The level of Histone H3 was used as a loading control.

(C) Light does not have a significant effect on BZR1 phosphorylation status. Phosphorylated (pBZR1) and unphosphorylated (BZR1) BZR1 was analyzed by immunoblotting using an anti-BZR1 antibody in *Arabidopsis* seedlings grown in dark (D), under red (R), or white (W) light for 3 (3 d) or 5 days (5 d). Seedlings grown in white light for 5 days were treated with 100 nM brassinolide (+BL) for 30 min. The gel blot was stained with Ponceau S to show protein loading (the Rubisco major band is weaker in dark-grown samples).

(D) A model for GATA2 function in BR- and light-regulation of photomorphogenesis. In the dark the BR-activated BZR1 directly represses *GATA2* transcription, and COP1 promotes GATA2 ubiquitination and degradation, ensuring a low GATA2 level for etiolation/skotomorphogenesis. In the presence of light, COP1 is inactivated, and the GATA2 protein accumulates to a high level to promote photomorphogenesis through binding to target genes. The GATA2 protein also feedback inhibits its own transcription by directly binding to its promoter, potentially desensitizing the system upon light-induced accumulation of GATA2 protein. When BR levels are low, reduced BZR1 activity leads to overexpression of GATA2, which promotes photomorphogenesis.

bound to the *DWF4* promoter strongly in both dark and light conditions. Such transcriptional regulation of *GATA2* leads to altered levels of GATA2 protein because BR treatment of the *det2* mutant dramatically reduced the GATA2 protein levels (Figure 6B). In contrast, BR and the BR biosynthetic inhibitor brassinazole had little effect on the GATA2 protein level in the transgenic plants that constitutively express *GATA2* from the 35S promoter (Figure S4), suggesting that BR represses GATA2 at the transcriptional but not posttranscriptional level.

Light Does Not Have a Strong Effect on BR Signaling

Our observation of differential BZR1 binding to the *GATA2* promoter in the dark and light suggests that light affects BZR1 activity. Because BZR1's nuclear localization and DNA-binding activity are tightly controlled by BR-regulated phosphorylation (Gendron and Wang, 2007), light could alter BZR1's phosphorylation status if light has an effect on BR level or BR signal transduction. Therefore, we performed immunoblotting experiments to test whether light affects BZR1 accumulation and phosphorylation (Figure 6C). The results show that plants grown in the dark and under red light or white light conditions contain similar levels of phosphorylated and unphosphorylated BZR1, whereas treatment with BR caused dramatic dephosphorylation of BZR1 (Figure 6C). These results indicate that light does not have a significant effect on BR level or BR signaling upstream of BZR1.

DISCUSSION

Interactions between light and endogenous hormones are critical for plant development. It has been long recognized that BR plays a major role in light-regulated plant development. The underlying molecular mechanism has remained unclear. This study identifies members of the GATA factor family (GATA2 and GATA4) as key transcription factors that integrate the BR and light-signaling pathways for coordinated regulation of gene expression and photomorphogenesis (Figure 6D). We show that GATA2 directly binds to light-responsive promoters in vivo and controls the expression of large numbers of genes that respond to both light and BR signaling. GATA2 is inhibited by BR signaling at the transcriptional level through BZR1 binding to its promoter and is activated by light at the protein level through inhibiting COP1-dependent proteolysis.

GATA2 also binds to its own promoter to feedback inhibit its own transcription. Such a feedback mechanism could serve as an important desensitizing mechanism during transition from dark to light, but it would also lead to de-repression of *GATA2*

transcription and incomplete switch to skotomorphogenesis when degradation of GATA2 is accelerated in the dark. As such, repression of *GATA2* expression by BR is essential for maintaining complete skotomorphogenesis in the dark. BR deficiency causes overexpression of *GATA2*, which contributes to de-etiolation in the dark. This study demonstrates a mode of BR-light crosstalk, in which BR signaling inhibits light responses through transcriptional repression of key components of the light-signaling pathway.

GATA2 Is a Key Component for Light-Responsive Gene Expression

Analyses of light-regulated promoters have shown an essential role of the GATA element in light-regulated gene expression (Chattopadhyay et al., 1998b; Jeong and Shih, 2003; Terzaghi and Cashmore, 1995). It has been shown that combinations of different LREs, rather than individual elements, confer proper light-responsiveness to a promoter (Puente et al., 1996). The GATA element functions together with the G-box or GT1 motifs to confer normal response to a wide spectrum of light signals involving multiple photoreceptors and the COP/DET/FUS complex (Chattopadhyay et al., 1998a). These results indicated a role of the GATA element as an essential partner with other LREs in light-regulated gene expression. Previous studies have only identified the G-box-binding factors, including PIFs, HY5, and CIB1. This study identifies GATA2 and GATA4 as the missing transcription factors that act through the GATA element.

Our results provide strong genetic and molecular evidence for the role of GATA2 in light regulation of gene expression and photomorphogenesis. First, overexpression of *GATA2* causes a typical de-etiolation phenotype and a transcriptomic change that resembles those caused by light exposure, whereas suppression of *GATA2* by RNAi or antisense had an opposite effect on hypocotyl elongation and gene expression. Although the long hypocotyl phenotypes of the *GATA-AM* and *-AS* plants are relatively weak, this is likely due to incomplete suppression of *GATA2* expression and/or redundant function of other homologous GATA factors. Second, ChIP assays showed that in vivo GATA2 binds to many light-responsive promoters at regions containing GATA motifs, providing direct evidence for GATA2 regulation of light-responsive genes. Finally, GATA2 protein is stabilized by light signaling, most likely through a COP1-dependent mechanism similar to the regulation of the light-signaling transcription factors HY5 and HFR1. GATA2 accumulates in the *cop1* mutants and can interact with COP1 and be ubiquitinated by COP1 in vitro, though direct in vivo interaction is yet to be demonstrated. Therefore, GATA2 meets the criteria for a primary light-signaling transcription factor.

GATA factors are a class of highly conserved transcription factors with a type IV zinc finger followed by a basic region, which are known to recognize the consensus sequence WGATAR (where W is T or A, and R is G or A) (Lowry and Atchley, 2000). GATA factors are found in all eukaryotes, including fungi, plants, and metazoans. In fungi, GATA factors are involved in a number of different processes, ranging from nitrogen utilization, mating-type switch, and light responses (Scazzocchio, 2000). In *Neurospora crassa*, the *White Collar-1* (*WC1*) and *White Collar-2* (*WC2*) loci encoding "plant-like" GATA factors are required for light and circadian responses (Ballario and Macino, 1997). In addition to the GATA DNA-binding domain, WC1 also contains a light-oxygen-voltage (LOV) domain and functions as a photoreceptor (Cheng et al., 2003). It seems that a function of GATA factors in light responses has been conserved during evolution from fungi to higher plants.

The function of *Arabidopsis* GATA factors in light response was not uncovered in previous genetic analysis, and suppression of *GATA2* and *GATA4* only partially suppressed *cop1* and *det2* mutants; these are most likely because of genetic redundancy. The *Arabidopsis* genome contains 29 genes that encode GATA factors (Reyes et al., 2004). Some members of the GATA family have been shown to play a role in regulating flower development (Zhao et al., 2004), chlorophyll synthesis, and carbon/nitrogen metabolism (Bi et al., 2005; Mara and Irish, 2008). In vitro DNA-binding assays have shown binding of GATA1 to the *GATA* elements of the *GAPB* promoter that are essential for light-responsive expression (Jeong and Shih, 2003). GATA2 shares 76% sequence identity with GATA1 in the DNA-binding domain and is likely to have similar DNA-binding specificity for GATA elements. Our ChIP experiment shows that GATA2 binds to promoter regions containing GATA sequence.

Additional GATA family members may be involved in light responses because their expression levels are regulated by light. Higher expression in the light-grown than dark-grown seedlings has been observed for *GATA6*, *GATA7*, *GATA21*, *GATA22*, and *GATA23* (Manfield et al., 2007). None of these genes is affected in the *GATA2-ox* plants based on our microarray data, suggesting that their light regulation is independent of GATA2. In fact *GATA21* and *GATA22* are induced by red light in a PIF3-dependent manner (Monte et al., 2004). In contrast, four other genes, *GATA2*, *GATA4*, *GATA9*, and *GATA12*, showed stronger expression in the dark-grown than light-grown seedlings (Manfield et al., 2007), and they are all repressed in the *GATA2-ox* plants. Based on similarity in sequence, gene structure, and expression profiles, these four *GATA* genes have been predicted to share common ancestry, with *GATA2* and *GATA4* arisen from a recent chromosomal duplication (Reyes et al., 2004). *GATA2* and *GATA4* are coexpressed with each other and share common coexpressed genes, which include PHYA and light-signaling transcription factors PIF3, PIF1/PIL5, and HFR1 (Manfield et al., 2007), consistent with their role in light signaling. In contrast, *GATA9* and *GATA12* do not show significant coexpression with any of the genes known to be involved in light signaling. It has been suggested that *GATA9* and *GATA12* have diverged from *GATA2* and *GATA4* in expression and possibly in function as well (Manfield et al., 2007). Based on our expression microarray data, only *GATA2* and *GATA4*, but not *GATA9* and *GATA12*, are overexpressed in the *bri1* mutant more than 2-fold and repressed in the *bri1 bzr1-1D* (Sun et al., 2010) double mutant, indicating that BR regulates the transcription of *GATA2* and *GATA4*, but not their close homologs *GATA9* and *GATA12*. Further genetic analysis of double or multiple loss-of-function mutants will be required to understand whether other GATA factors also play a role in photomorphogenesis.

The relationship between GATA2 and other light-signaling transcription factors is key for understanding light-responsive gene expression. Several lines of evidence suggest that GATA2 functions together with the G-box-binding factors. First, GATA and G-box elements are found together in many

light-responsive promoters, and their dual presence is essential for normal light responsiveness in a synthetic promoter (Chattopadhyay et al., 1998b). Second, *GATA2* shows strong coexpression with *PIF3*, *PIF1*/*PIL5*, *SPT*, and *HFR1* (Manfield et al., 2007), many of which bind to the G-box. Third, GATA2 is stabilized by light at the posttranslational level, likely through the same COP1-dependent mechanism that regulates HY5 and HFR1. It is also worth noting that a higher percentage of the genes upregulated than downregulated in *GATA2-ox* are HY5 targets (Lee et al., 2007) (27% of 1167 upregulated genes versus 21% of 1743 downregulated genes), which is consistent with our hypothesis that GATA and G-box elements together confer light-activated expression by recruiting GATA2 and HY5. Whether GATA2 directly interacts with other light-signaling transcription factors and how they orchestrate dynamic light-regulated gene expression are yet to be analyzed in future studies.

GATA2 Is a Key Junction for the Antagonism between BR- and Light-Signaling Pathways

Genetic studies have long demonstrated a critical role of BR in skotomorphogenesis (Li et al., 1996; Szekeres et al., 1996). The antagonizing relationship between BR and light has been analyzed at the genetic and physiological levels. Mutations that reduce BR level enhanced the light responses (Neff et al., 1999), and a rice *phyB* mutant showed enhanced BR responses (Jeong et al., 2007). The antagonism at the level of gene expression was recognized in the initial studies of the BR-deficient mutants (Chory et al., 1991) (Li et al., 1996; Szekeres et al., 1996) and confirmed by our microarray data showing similar transcriptomic changes caused by the *bri1* mutation and light exposure. The similar effects of BR deficiency and light on seedling development and expression of large numbers of genes suggested three possible mechanisms of interaction between the BR- and light-signaling pathways: (1) light reduces BR level or BR sensitivity, (2) BR regulates light-signaling components to inhibit light signaling, or (3) BR- and light-signaling pathways regulate common target genes through separate transcription factors independently controlled by each pathway. This study provides evidence for the second mechanism of BR-light crosstalk, and recent genomic analysis of BZR1 target genes supported the presence of also the third mechanism (Sun et al., 2010).

A previous study proposed that light inhibits BR biosynthesis by repressing a small G protein that binds to and activates a BR-biosynthetic enzyme (Kang et al., 2001). However, subsequent direct BR measurement failed to detect significant difference in BR levels between light-grown and dark-grown plants but showed light reducing the level of gibberellin, another hormone that also promotes cell elongation (Symons and Reid, 2003). Our observations of no obvious effect of light on the phosphorylation status and accumulation of BZR1 or on BZR1 binding to the *DWF4* promoter are consistent with the lack of change of BR level by light. Our results further suggest that light does not inhibit BR signaling upstream of BZR1. However, stronger BZR1 binding to the *GATA2* promoter was observed in the dark-grown than light-grown seedlings. It is possible that light has an effect on the availability of BZR1-binding site or BZR1-interacting proteins at the *GATA2* promoter. In contrast to the lack of strong effect of light on BR signaling, BR obviously has a strong effect on light signaling by repressing *GATA2* expression.

Our results show that GATA2 plays a key role in BR regulation of photomorphogenesis. GATA2 accumulates in the *det2* mutant, and GATA2 knockdown partially suppresses the photomorphogenic phenotypes of dark-grown *det2* and *bin2*, indicating that de-etiolation in the BR mutants is at least partly due to the increased levels of *GATA2*. About one-third of the genes affected in *bri1* are affected similarly by *GATA2-ox*, suggesting that the elevated GATA2 level contributes to a major portion of *bri1*'s effect on genome expression and that BZR1 repression of *GATA2* is a major mechanism for BR inhibition of light responses. By inhibiting transcription and promoting protein accumulation of GATA2, respectively, BR and light antagonistically regulate the level of GATA2 activity and, consequently, the expression of its downstream target genes. Thus, GATA2 represents a key junction of crosstalk between BR- and light-signaling pathways.

The mechanism of BR-light crosstalk through GATA2 is distinct from those for light crosstalk with GA and cytokinin. In addition to light repression of GA level, GA also affects the activity or accumulation of the light-signaling transcription factors PIF/PIL and HY5 (Alabadi et al., 2008; de Lucas et al., 2008; Feng et al., 2008). The DELLA proteins of the GA-signaling pathway directly interact with and inhibit members of the PIF/PIL family, which are negative regulators of photomorphogenesis (de Lucas et al., 2008; Feng et al., 2008). GA also promotes degradation of HY5, possibly through a COP1-dependent process (Alabadi et al., 2008). In contrast, cytokinin, which promotes photomorphogenesis, induces HY5 protein accumulation (Vandenbussche et al., 2007). Whether other hormones also regulate GATA2 to modulate light responses remains to be tested by future studies.

EXPERIMENTAL PROCEDURES

Plant Materials and Growth Conditions

The WT, various mutants, and transgenic *Arabidopsis thaliana* plants were in the *Columbia* ecotype. Seeds were sterilized by incubation in freshly prepared 10% bleach plus 0.01% Triton X-100 for 15 min and then washed three to four times with sterilized water. The surface-sterilized seeds were treated in 4°C for 2 days and at 22°C under white light for 8 hr to induce uniform germination. For phenotype analyses, seedlings were grown on 0.8% phytoagar plates containing half-strength Murashige-Skoog (MS) nutrient and 1% sucrose. White light (about 100 $\mu mol/m^2/s$) was provided by fluorescence light source in a growth room at 22°C. Growth under red, far-red, and blue light was carried out in a LED light chamber (E-30LEDL3, Percival) at 22°C. Seedlings were photographed next to a size reference (ruler) and their hypocotyl lengths measured using the ImageJ software. Seeds were harvested from plants grown in a greenhouse supplemented to 16 hr light/day and a temperature range of 18°C–28°C.

Vector Construction and Transformation

A 1152 bp genomic fragment containing full-length *GATA2* open reading frame was amplified by PCR and then cloned into the BamHI and KpnI sites of the pSN1301 binary vector to place *GATA2* under the control of the CaMV 35S promoter.

The *GATA2-AS* construct was made by inserting the *GATA2* full-length cDNA fragment in reverse orientation into the pSN1301 plasmid. The artificial microRNA constructs were made using the vectors and methods previously reported (Schwab et al., 2006) (see Supplemental Experimental Procedures for details). The *35S::GATA2-YFP* fusion construct was generated by inserting

a full-length *GATA2* cDNA without stop codon fused to the N terminus of the pEZR-LNY vector.

The *GATA2-ox*, *GATA2-AS*, *GATA2-AM*, and *35S::GATA2-YFP* binary constructs were transformed into the *Agrobacterium tumefaciens* strain GV3101 and then introduced into *Arabidopsis thaliana* Columbia WT plants via a floral dip method. About 20 T1 transgenic lines with single T-DNA insertion were selected for further analysis. Homozygous T3 or T4 transgenic seedlings were used for phenotype and molecular characterization.

Protein Expression and Antibody Preparation

The full-length GATA2 cDNA was cloned into the pGEX-4T-1 vector to express GST-GATA2 protein in *E. coli* Rosetta cells (Novagen). The recombinant fusion protein was purified using glutathione-agarose beads (GE Healthcare) and used to immunize rabbit. The anti-GATA2 antibody was purified from the immune serum using immobilized GST-GATA2 (Aminolink® Immobilization Kit, Pierce Biotechnology). The anti-Histone H3 antibody for loading control was from Millipore (catalog number 07-690).

Total RNA Isolation and Quantitative RT-PCR Analysis

Total RNA was extracted from *Arabidopsis* seedlings using the TRIzol RNA extraction kit (Invitrogen, USA). The first-strand cDNA was synthesized by using M-MLV reverse transcriptase (Promega, USA) and used as RT-PCR templates. Quantitative real-time PCR analyses were carried out on Mx3000P (Stratagene, USA) by using the SYBR® Green reagent (Toyobo, Japan) according to the manufacturer's instructions. The RT-PCR was repeated at least three times using samples harvested separately. The *UBC30* gene was used as internal reference. See Supplemental Experimental Procedures for primer sequences used for RT-PCR.

Microarray Data Analysis

Arabidopsis seedlings (Columbia, *GATA2-ox*, *bri1-116*) were grown on 1/2 MS medium in the dark for 4.5 days, and the seedlings were frozen in liquid nitrogen in complete darkness, and then the *bri1-116* seedlings were selected from the segregating population. Ten micrograms (10 μg) of total RNA from the seedlings was used to prepare probes for hybridization, and each probe was hybridized independently to one chip according to the protocol of the ATH1 array manufacturer (Affymetrix). Three independent biological repeats were conducted. The data were analyzed using Genespring software ver. 7. Data that were flagged as absent, using the Affymetrix mismatch probes, in two or more of the repeats for each genotype were removed. Genes that passed this filter for any one of the genetic backgrounds were used for further analysis. P value <0.05 and fold change >2 (for *GATA2-ox*) or fold change >1.8 (for *bri1-116*) were used to identify genes differentially expressed in *GATA2-ox* or *bri1-116* compared to WT control seedlings.

To determine what experimental conditions cause similar gene expression changes as *GATA2-ox*, we carried out expression fingerprint searching by comparing the differential gene expression pattern between *GATA2-ox* treatment and all available 1450 treatment/control microarray comparisons (T/Cs) in the Gene Expression Browser (GEB) database (http://www.expressionbrowser.com/) (Zhang et al., 2010). We inputted the pairs of *GATA2-ox* significant (2-fold and $p < 0.05$ as cutoff) gene IDs and their log2 ratios, and compared them to each T/C of GEB with the following procedure: (1) Select the significant genes from the T/C using 2-fold and $p < 0.05$ as cutoff. (2) Compute the overlapping genes between *GATA2-ox* and the T/C. The chi-square test was used for filtering out nonsignificant overlaps ($p < 0.01$ as cutoff). (3) Compute the Pearson's correlation coefficient using the paired log2 ratios of *GATA2-ox* and the T/C for the overlapping genes. The significance of correlation P value was also computed to reject nonsignificant correlations ($p < 0.01$ as cutoff). As a result, all hits were significant in both the number of overlapping genes and expression changes (Pearson correlation). Finally, the hit list was ordered by the Pearson correlation coefficient.

ChIP

ChIP experiments were performed following the protocol described previously (He et al., 2005) using 2-week-old light-grown WT and *35S::GATA2-YFP* transgenic *Arabidopsis* seedlings or 5-day-old dark- and light-grown 35S-GFP and *pBZR*::BZR1-CFP seedlings. An affinity-purified anti-GFP polyclonal antibody was used to immunoprecipitate the BZR1 or GATA2 protein-DNA complex, and the precipitated DNA was analyzed by real-time PCR using the SYBR® Green reagent. Results were presented as the ratio of the amount of DNA immunoprecipitated from BZR1-CFP or GATA2-YFP samples to that of the control samples (35S-GFP or WT). The *UBC30* and *PP2A* genes were used as the negative controls. The ChIP experiments were performed three times, from which the means and standard deviations were calculated. The primer sequences for ChIP-qPCR are in Supplemental Experimental Procedures.

Protein Purification and Pull-Down Assay

The GST-GATA2 protein was expressed using the pGEX-4T-1 vector in *E. coli* Rosetta cells. The recombinant fusion protein was purified using glutathione-agarose beads. For pull-down assay, COP1 fused to maltose binding protein (MBP) was purified using amylose resin (NEB). Glutathione beads containing GST-GATA2 were incubated with MBP, MBP-COP1. The mixture was rotated in a cold room for 1 hr, and the beads were washed five times with wash buffer (20 mM Tris-HCl [pH8.0], 200 mM NaCl). The proteins were eluted from the beads by boiling in equal volume of 2× SDS buffer and loaded onto a SDS-PAGE gel. Gel blots were analyzed using an anti-MBP antibody (NEB).

In Vitro Ubiquitination Assay

The MBP-COP1 and GST-GATA2 proteins expressed in *E. coli* were affinity purified for in vitro ubiquitination assays. To improve the E3 activity of MBP-COP1, the purified MBP-COP1 and MBP control proteins on maltose beads were incubated with *Arabidopsis* cell extract for 30 min. After incubation, the cell extract was removed, and the beads were washed. To perform the in vitro ubiquitination assay, crude extracts containing recombinant wheat E1 (GI: 136632) were incubated with human E2 (UBCh5b), His-UBI (UBQ14), purified GST-GATA2, and purified MBP-COP1 (or MBP control) at 30°C with agitation in an Eppendorf Thermomixer for 1.5 hr. The proteins were immunoblotted after SDS-PAGE, and GST-GATA2 was detected using an anti-GST antibody.

ACCESSION NUMBERS

The microarray data have been deposited in the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) with series accession number GSE25396.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, four figures, and four tables and can be found with this article online at doi:10.1016/j.devcel.2010.10.023.

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