Control of Specific Gene Expression by Gibberellin and Brassinosteroid[1]

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We identified a recessive, brassinolide-insensitive mutant caused by a deletion allele (*bri1-201*) of the brassinosteroid (BR) receptor *BRI1*. The *bri1-201* mutant displayed altered expression levels of genes differentially regulated by gibberellin (GA). RNA-blot analysis revealed that BR and GA antagonistically regulate the accumulation of mRNAs of the GA-responsive *GASA1* gene, as well as the GA-repressible *GA5* gene. Expression studies with cycloheximide indicated that the antagonistic effects of GA and BR on *GA5* require de novo protein synthesis. Reporter transgene analyses and RNA-blot analysis showed that BR and GA modulate *GA5* expression, at least in part, at the transcriptional level, and that the signals are independent and subtractive.

Brassinosteroids (BR) and gibberellins (GA) are plant growth regulators controlling cell and plant size, and mutations impairing their biosynthesis or sensitivity result in dwarfism. Early physiological work on wild-type (WT) tissues of different plants showed that GA and BR additively enhance growth, indicating that the two hormones act independently at the cellular level (Gregory and Mandava, 1982). However, recent molecular work indicates that cross-talk may occur between BR- and GA-signaling pathways. For example, mRNA of the GA-responsive *γ-TIP* gene accumulates ectopically in BR-deficient and BR-signaling mutants, suggesting that BR and GA antagonistically regulate *γ-TIP* expression (Kauschmann et al., 1996). *γ-TIP* encodes a tonoplast-intrinsic aquaporin or water channel, and its antagonistic regulation by BR and GA may reflect differences in the mechanisms by which the two hormones modulate cell growth and size by regulating turgor pressure or solute flow. In contrast, mRNA levels of the *MERI-5* gene (Medford et al., 1991) are regulated positively by either BR or GA treatment (Kauschmann et al., 1996). *MERI-5* probably encodes a xyloglucan-endohydrolase involved in cell wall loosening, thereby modulating cell expansion and growth. If so, *MERI-5* expression may be required for growth processes mediated by both GA and BR.

Feedback control of the expression of BR and GA biosynthetic genes regulates endogenous levels of the growth hormones. For example, BR negatively controls transcription of the *CPD* steroidogenic cytochrome P450 (Mathur et al., 1998). In a similar manner, GA negatively regulates the expression of the *GA4* 3β-hydroxylase (Chiang et al., 1995) and the *GA5* GA 20-oxidase-1 (Phillips et al., 1995; Xu et al., 1995), which encode enzymes converting inactive GA precursors into active GAs. If cross-talk occurs between GA and BR signaling, biosynthetic enzymes such as CPD, GA4, and GA5 may be potential regulatory targets. Such cross-talk could occur via shared signaling components, interactions between components specific for each pathway, or via control of the transcription or stability of common targets by distinct factors.

During the course of a phenotypic screen of gamma-mutagenized plants, we isolated a dwarf mutant similar to GA or BR biosynthetic/signaling mutants. Here, we demonstrate that this mutant phenotype is caused by a novel deletion allele (*bri1-201*) of the BRI1 BR receptor (Li and Chory, 1997; He et al., 2000). RNA-blot analysis with RNAs from WT, BR-insensitive *bri1-201*, and BR-deficient *cpd* mutants showed that BR and GA antagonistically regulate *GASA1* and *GA5*. Expression studies with the protein synthesis inhibitor cycloheximide (CHX) indicated that *GA5* regulation by BR or GA requires de novo protein synthesis. Transgene reporter analysis and RNA-blot analysis also indicated that *GA5* regulation by BR and GA occurs, at least in part, at the transcriptional level.

RESULTS

The *bri1-201* Mutant and Allele

A screen of 100,000 progeny from gamma-mutagenized plants for visible mutant phenotypes identified the dwarf shown in Figure 1. This mutant exhibited reduced size at early stages of develop-

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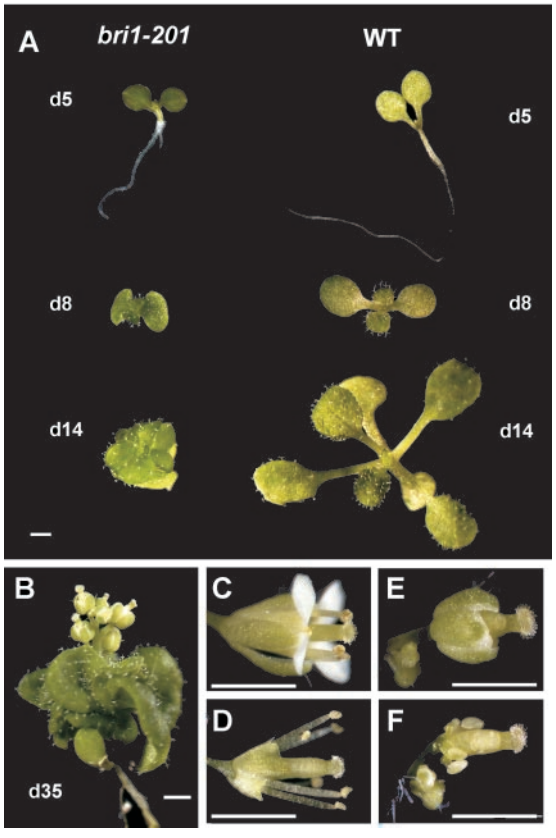

Figure 1. Phenotype of the *bri1-201* mutant under long-day conditions (16 h light/8 h dark). A, Comparative development of *bri1-201* and WT. B, *bri1-201* 35 d after germination. C through F, WT (C and D) and *bri1-201* flowers (E and F). In D and F, sepals and petals were removed to show reproductive organs. Bars represent 1 mm.

ment, reduced apical dominance, extreme dwarfism at flowering, and delayed flowering and leaf senescence (Fig. 1, A and B). The mutant was apparently male sterile (Fig. 1, E and F) because homozygous seed was not produced by selfing, although pollination of the dwarf with WT pollen produced viable seed. F_2 progeny from such crosses segregated in a 3:1 ratio (210 mutants out of 949 plants, $\chi^2 = 0.04$), indicating that the mutant phenotype was caused by a single recessive allele. The phenotype of the mutant suggested that it might be the result of a lesion in the biosynthesis or sensitivity to growth hormones such as BR or GA.

We used two approaches to elucidate the cause of the mutant phenotype: physiological and molecular studies of BR and GA responses and physical mapping. To investigate sensitivity to BR, the dwarf, as well as WT, BR-deficient *cpd* (Szekeres et al., 1996), and BR-insensitive *bri1-1* (Clouse et al., 1996) controls were grown on Murashige and Skoog medium for 2 weeks and then transferred to plates supplemented with 1 μM 24-epibrassinolide (EBR). WT and *cpd* plantlets exhibited BR-sensitive growth, whereas the dwarf and *bri1-1* failed to normalize leaf development (Fig. 2A). When grown on EBR concentrations inhibiting WT root elongation, both the dwarf and *bri1-1* seedlings also maintained equivalent root growth (Fig. 2B). Moreover, dark-grown dwarf and *bri1-1* seedlings were shorter than WT and both exhibited skotomorphogenesis, a characteristic of BR mutants (Fig. 2C). Taken together, these results indicate that the dwarf is BR insensitive.

The dwarf locus was mapped by scoring F_2 mutant progeny, from a cross between plants heterozygous for the dwarfing allele (Columbia-0 [Col-0] ecotype) to WT Landsburg *erecta*, for segregation of using simple sequence-length polymorphism markers spanning the 10 Arabidopsis chromosome arms (Bell and Ecker, 1994). The mutation thus was localized to the lower arm of chromosome IV, south of marker nga1107 (three recombinants out of 60 mutant plants examined). Because *BRI1* maps in this region (Li and Chory, 1997), this suggested that the dwarf might be caused by an allele of *bri1*. To test this, the *BRI1* genes from three dwarf plants were PCR amplified and sequenced. An 8-bp deletion was found in all three mutants 22 nucleotides downstream of the start ATG, which produced a frame shift in the open reading frame resulting in a stop codon after 44 amino acid residues. Therefore, we designated this allele and the mutant *bri1-201*. RNA-blot analysis showed that WT

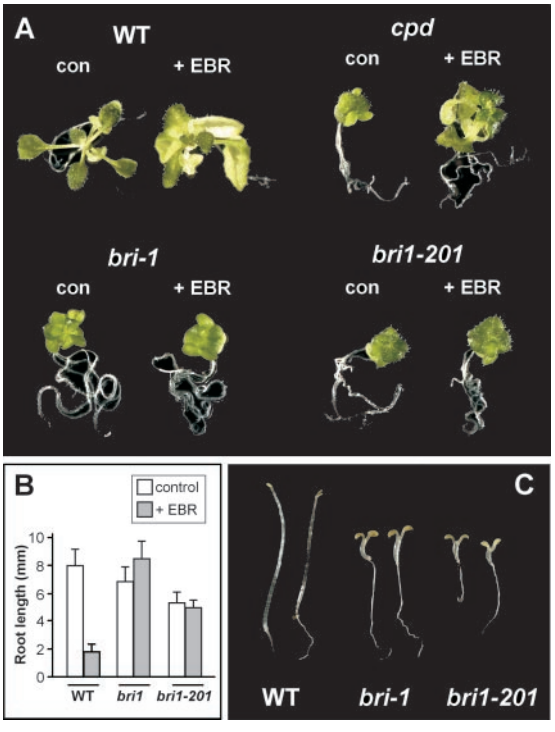

Figure 2. *bri1-201* exhibits insensitivity to EBR and constitutive skotomorphogenesis. A, Two-week-old WT and *bri1-201* were transferred to plates without (con) or with (+EBR) 0.5 μM EBR and grown for 5 d. B, Root length of WT, *bri1-1* and *bri1-201* seedlings grown 5 d without (control) or with (+EBR) 0.2 μM EBR. Each value represents the mean of 50 independent measurements. C, Skotomorphogenesis of WT and mutant seedlings. Seedlings were germinated and grown for 5 d in darkness on Murashige and Skoog medium.

and *bri1-201* plantlets accumulated equivalent levels of mRNA hybridizing to a *BRI1* 3′-untranslated region probe (data not shown). This indicates that transcription and stability of *BRI1* mRNA are not affected by BR levels or BR-signaling intermediates in WT Arabidopsis plants. Because *bri1-201* lacks any BRI1 protein and is slightly more dwarfed than *bri1* (Fig. 2, B and C), *bri1-201* constitutes a null allele similar to the deletion mutant *bri1-4*, which exhibits a frame shift in the BRI1 open reading frame at 140 amino acid residues (Noguchi et al., 1999).

Expression of GA-Regulated Genes in *bri1-201*

Our interest in plant responses to GAs (Raventos et al., 2000; Meier et al., 2001) initially prompted us to examine whether the mutant was sensitive to the application of exogenous GA prior to our identification of the *bri1-201* allele. Growth assays with seedlings and rosette-stage plants showed that aerosol treatment with 10^{-5} M GA_3 did not restore WT growth to the mutant, suggesting that it might be insensitive to GA. We attempted to confirm this by examining the expression of several GA-regulated genes in the mutant by RNA-blot analysis. These included the GA-responsive *GASA1* gene (Herzog et al., 1995), the GA feedback-regulated, biosynthetic genes *GA5* and *GA4* (Chiang et al., 1995; Phillips et al., 1995; Xu et al., 1995), and the *RGA* and *GAI* genes encoding GA-signaling components (Peng et al., 1997; Silverstone et al., 1998). This revealed that differential gene expression by GA was not affected in the mutant because accumulation of *GASA1*/*GAI*/*RGA* was enhanced by GA_3, whereas accumulation of *GA5*/*GA4* mRNAs was still negatively feedback regulated by GA_3 (Fig. 3). This indicated that the mutant was sensitive to GA because it was not qualitatively compromised in responses to GA at the molecular level. Nonetheless, we noted that the control mRNA levels of *GASA1* and *GA5* were significantly different between the mutant and WT. For example, *GA5* mRNA was three times less abundant in *bri1-201* than in WT, whereas *GASA1* mRNA was more than 7-fold higher in the mutant. Moreover, the levels of *GA4*, *RGA* and *GAI* mRNAs were slightly lower in *bri1-201* than in WT.

These results would be consistent with a lesion affecting the amplitude of GA-dependent responses controlling the expression of both GA inducible and repressible genes such as *GASA1* and *GA5*. However, our subsequent finding that the mutant is BR insensitive indicated that BR affects the expression of these genes antagonistically to GA. This would explain why *GA5* mRNA levels are lower, whereas *GASA1* levels are higher in the *bri1-201* mutant than in WT. In an alternate manner, the alteration in *GASA1* and *GA5* mRNA accumulation levels observed in *bri1-201* could be due to pleiotropic effects of the mutation.

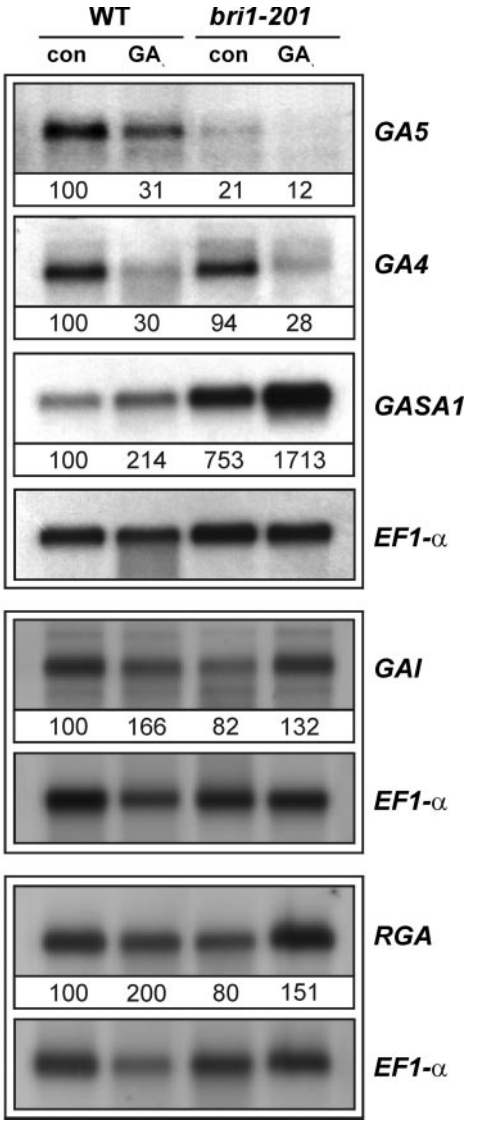

Figure 3. RNA-blot analysis of *GA5*, *GA4*, *GASA1*, *GAI*, and *RGA* mRNA accumulation in *bri1-201* and WT upon GA_3 treatment. Poly(A^+) RNA (1 μg lane^{-1}) from 16-d-old plants grown on Murashige and Skoog medium without or with 50 μM GA_3 added for the last 48 h. Ribonucleic ^{32}P-CTP antisense probes were synthesized using T7 RNA polymerase from partial cDNA 3′ sequences cloned in the pGEM-Teasy vector. *GA5*, *GA4*, and *GASA1* hybridizations were performed on the same filter, whereas *GAI* and *RGA* hybridizations were performed on independent filters. Radioactive signals were quantified on all membranes and standardized (WT con = 100) by comparison to signals obtained after subsequent blotting with the *EF1-α* probe.

Effect of BR and GA on *GA5* and *GASA1* Steady-State mRNA Levels

To investigate whether altered GA-related gene expression in *bri1-201* is correlated to deficiencies in BR signaling, EBR treatments were performed on WT, *bri1-201*, and the BR-deficient *cpd* mutant (Szekeres et al., 1996). mRNA levels were again monitored by

EF1-α-normalized RNA-blot analysis. This showed that EBR treatment increased *GA5* mRNA slightly in WT and more strongly in the BR-deficient *cpd* mutant, but did not affect *GA5* mRNA levels in *bri1-201* (Fig. 4A). In contrast, accumulation of the GA responsive *GASA1* mRNA was reduced by EBR slightly in WT and very strongly in *cpd*. These results indicate that BR and GA antagonistically affect *GA5* and *GASA1* mRNA levels.

Although *GA4* and *RGA* mRNA levels were somewhat lower in *bri1-201* and *cpd* than in WT, EBR treatment failed to restore their *mRNA* levels in the *cpd* mutant, in contrast to *GA5* (Fig. 4A). This result indicates that BR effects on *GA4* expression are less pronounced than BR control of *GA5*.

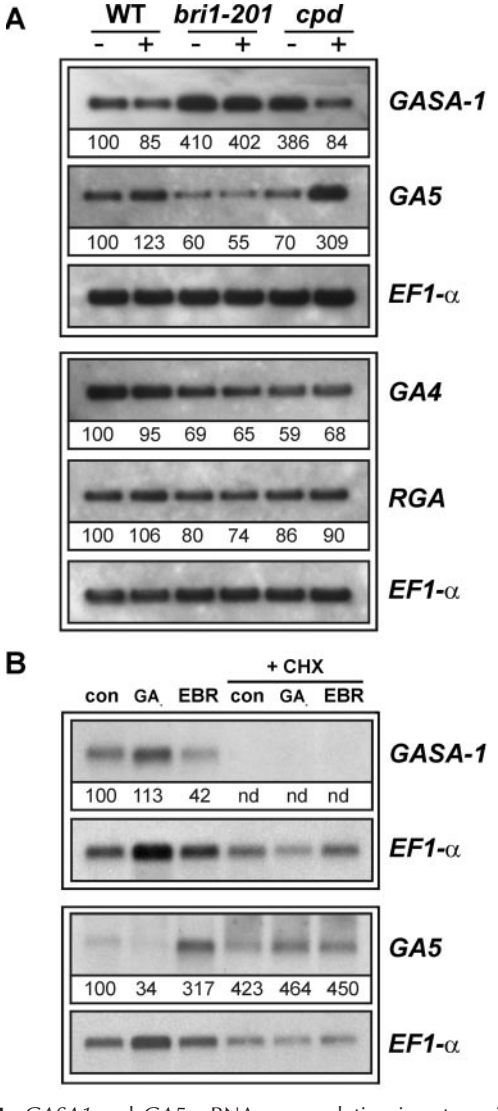

Figure 4. *GASA1* and *GA5* mRNA accumulation is antagonistically controlled by GA and BR. Two-week-old seedlings grown on Murashige and Skoog were transferred to 50 mL liquid one-half-strength Murashige and Skoog in flasks for 1 d prior to treatment. RNA-blot analyses were performed and normalized with *EF1-α* as described in Figure 3. A, WT, *bri1-201* and *cpd* seedlings were treated with 1 μM EBR for 48 h. B, *cpd* seedlings were treated with GA or BR in presence (CHX) or not of translational inhibitors. CHX (50 μM) and chloramphenicol (50 μM) were added to the medium 2 h before GA or BR treatments (50 μM GA_3 or 1 μM EBR for 16 h).

To assess whether de novo protein synthesis affects the accumulation of steady-state mRNA levels of *GASA1* and *GA5*, GA and BR treatments were performed in the presence of translational inhibitors, and mRNA levels monitored by *EF1-α*-normalized RNA-blot analysis. As shown in Figure 4B (top, lanes 1 and 2 versus lanes 4 and 5), CHX blocked both basal levels of *GASA1* mRNA accumulation in control tissue, as well as the enhancement of *GASA1* mRNA accumulation by GA treatment. This suggests that GA-responsive *GASA1* mRNA accumulation requires the de novo synthesis of an activator or a factor that stabilizes *GASA1* mRNA. However, because CHX abolished and BR treatment decreased *GASA1* mRNA accumulation, these experiments do not indicate whether BR repression of *GASA1* mRNA accumulation requires de novo protein synthesis.

In contrast to *GASA1*, RNA-blot analysis showed that CHX alone induced *GA5* mRNA accumulation to above-control levels, and that CHX blocked the repression of *GA5* accumulation by GA (Fig. 4B; bottom, lane 2 versus lane 5). This is consistent with a model in which a labile repressor is required for GA negative feedback regulation of *GA5* (Meier et al., 2001). However, BR induction apparently was blocked by CHX because *GA5* mRNA levels were similar following treatment with CHX alone, or CHX together with GA or BR (Fig. 4B; bottom, lanes 4–6). These results do not distinguish between whether BR enhancement of *GA5* mRNA accumulation is effected via a de novo-synthesized activator or an mRNA-stabilizing factor, or via derepression of GA negative feedback regulation.

BR Regulation of the *GA5* Promoter

We previously have generated transgenic plants expressing the firefly luciferase (*LUC*) gene under the control of 0.4 kb of the *GA5* promoter, and demonstrated that this promoter fragment contains cis sequences required for transcriptional regulation of *GA5* by GA (Meier et al., 2001). This *GA5-LUC* reporter was introduced into the BR-deficient *cpd* mutant by crossing. F_3 seedlings, homozygous for the *GA5-LUC* reporter and exhibiting either WT or *cpd* phenotypes, were treated with EBR and their in vivo LUC expression measured with a CCD camera. EBR treatment of WT plants carrying *GA5-LUC* did not significantly affect LUC reporter activity, although the control level of LUC activity was 3-fold higher in these WT plants than in the *cpd* plants expressing the reporter (Fig. 5A). This result is consistent with the low *GA5* transcript accumulation level observed in *bri1-201*, and suggests that endogenous BR levels in the WT contribute to basal *GA5* expression levels. In contrast, expression of

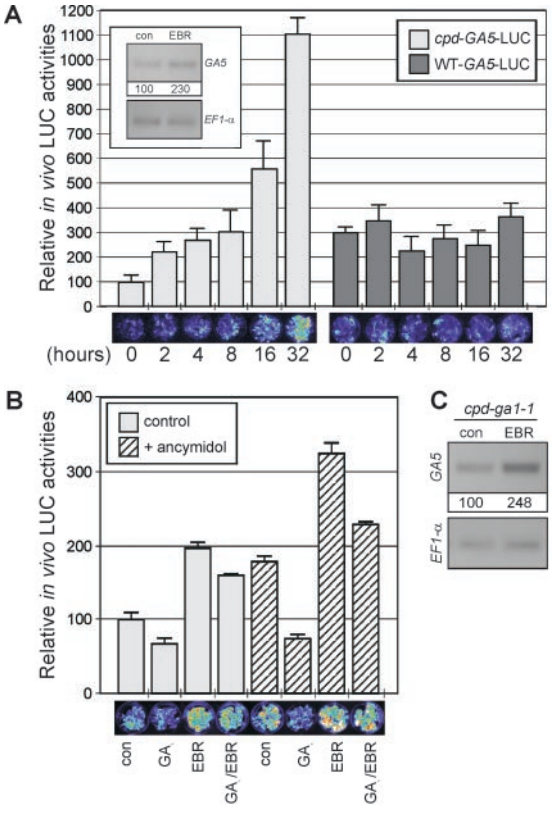

Figure 5. Effects of BR and GA on *GA5-LUC* expression. A, LUC imaging of the *GA5-LUC* reporter in 16-d-old WT-*GA5-LUC* and *cpd-GA5-LUC* seedlings treated with 1 μM EBR for various times. Insert in A, *GA5* transcript accumulation monitored by RNA-blot analysis from 16-d-old *cpd* seedlings treated with 1 μM EBR for 2 h. Hybridization conditions and normalization with *EF1-α* were performed as described in Figure 3. B, One-week-old *cpd-GA5-LUC* seedlings grown on Murashige and Skoog plates were transferred to liquid one-half-strength Murashige and Skoog in presence or absence of ancymidol (1 mg L^{-1}) and grown for 7 more d. BR (0.1 μM EBR) and GA (50 μM GA_3) treatments were performed for 16 h by adding the hormones to the medium. C, *GA5* transcript accumulation in 16-d-old *cpd-ga1-1* double mutant seedlings treated with 1 μM EBR for 16 h. Hybridization conditions and normalization with *EF1-α* were performed as described in Figure 3. In A and B, bioluminescent LUC images (displayed in pseudocolors) acquired by CCD camera are shown below the quantification (average gray values) of LUC images.

the *GA5-LUC* reporter in *cpd* plants increased after 2 h of BR treatment and reached up to 11-fold induction after 32-h treatment (Fig. 5A). This indicates that the *GA5* gene is, at least in part, transcriptionally regulated by BR, and in an opposite manner to GA. Accumulation of *GA5* transcript in *cpd* seedlings was monitored by RNA-blot analysis after a short-term treayment (2 h) with EBR and confirmed the rapid regulation of *GA5* transcript levels by BR observed in the reporter assays (Fig. 5A, inset).

If GA and BR antagonistically regulate *GA5* transcription via independent pathways, the two signals may be subtractive. To investigate this possibility, *cpd-GA5-LUC* seedlings were subjected to GA_3 and EBR treatments in the presence or absence of the GA biosynthesis inhibitor ancymidol (Rademacher, 1989). As expected, GA_3 treatment down-regulated *GA5-LUC* activity, whereas EBR treament up-regulated reporter activity (Fig. 5B, bar 1 versus bars 2 and 3). In the presence of both hormones, LUC activity was reduced to the same extent in WT and WT treated with GA_3 (Fig. 5B, bars 1 and 2 versus bars 3 and 4). As we have observed previously (Meier et al., 2001), LUC reporter activity was also up-regulated by ancymidol treatment (bar 1 versus bar 5). This indicates that the basal levels of active GAs in seedlings contribute to negative feedback of the *GA5* gene. It is interesting that reporter activity was increased to the same extent upon EBR treatment of *cpd* seedlings grown in the presence or absence of ancymidol (Fig. 5B, bars 1 and 3 versus bars 5 and 7). Moreover, in the presence of ancymidol, GA_3 reduced reporter activity to the same extent in plants treated with (Fig. 5B, bar 7 versus bar 8) or without (bar 5 versus bar 6) EBR.

To assess the specificity of the ancymidol treatment on the inhibition of GA biosynthesis, and to rule out the possibility of side effects on other hormone biosynthetic pathways (i.e. BR), we generated *cpd-ga1-1* double mutants in which both active GA and BR levels are dramatically reduced. Accumulation of *GA5* mRNA upon EBR treatment in the *cpd-ga1-1* double mutant was investigated by means of RNA-blot analysis. As shown in Figure 5C, EBR treatment induced *GA5* mRNA accumulation in *cpd-ga1-1* seedlings, indicating that BR-induced *GA5* expression is GA independent. This result is consistent with the additivity of the EBR and ancymidol treatments on the *GA5-LUC* reporter activation observed in Figure 5B. Taken together, these results show that GA and BR modulate *GA5* transcription in a subtractive manner.

DISCUSSION

Dwarfism is a commonly identified mutant phenotype and can result from lesions in phytohormone synthesis or sensitivity (Li and Chory, 1997; Peng et al., 1997; Johnson and Ecker, 1998), or in potentially pleiotropic stress signaling (Bowling et al., 1994). Therefore, we undertook two approaches to determine the lesion causing dwarfism in a mutant identified in a phenotypic screen of gamma-mutagenized seeds. First, map-based cloning and genomic sequencing identified the mutation as a novel deletion allele (*bri1-201*) of the *BRI1* gene encoding the BRI1 BR receptor (Li and Chory, 1997; Friedrichsen et al., 2000; He et al., 2000). Second, physiological analyses showed that *bri1-201* is insensitive to BR, as expected. In addition, and prior to our identification of the *bri1-201* allele, our interest in GA action (Raventos et al., 2000; Meier et al., 2001) prompted us to examine

GA responses at the molecular level in *bri1-201*. RNA-blot analysis revealed that mRNA levels of GA-inducible *GASA1* (Herzog et al., 1995) were higher, whereas mRNA levels of GA-repressible *GA5* (Phillips et al., 1995; Xu et al., 1995) were lower in *bri1-201* compared with WT. These results indicated that although *bri1-201* is qualitatively sensitive to GA, the expression of specific genes is antagonistically affected by *BR* and *GA*. This was confirmed by transgenic experiments showing that expression of a fusion between the *GA5* promoter and the *LUC* reporter was antagonistically regulated by GA and BR in *cpd* seedlings. In addition, endogenous *GA5* up-regulation was observed in *cpd-ga1-1* double mutants upon EBR treatment, confirming that BR and GA effects on *GA5* expression are independent and subtractive.

These results indicate that cross-talk exists between these two important growth hormones, and that GA and BR modulate the expression of *GA5*, at least in part, at the transcriptional level. A result of this cross-talk may be that BR potentiates GA action by positively affecting *GA5*, a key GA biosynthetic enzyme whose activity regulates active GA levels (Phillips et al., 1995; Xu et al., 1995). This is consistent with phenotypic studies of the BR-deficient *dwf4* mutant showing that a fully active BR pathway is required for cell elongation as a response to GA (Azpiroz et al., 1998). Using reporter transgenes, we have observed that *GA4* is expressed in the hypocotyl of young seedlings (data not shown), which is also the case for *GA1* (Silverstone et al., 1997) and for *GA5* (Meier et al., 2001). This suggests that active GAs are produced in the hypocotyl. These data are consistent with the results of (Ephritikhine et al., 1999) showing that in the BR-deficient *sax1* mutant, GA-insensitive cell elongation is restricted to the hypocotyl and is reversible by BR application. Moreover, Goetz et al. (2000) have recently shown that the *Lin6* gene, encoding an extracellular invertase responsible for phloem unloading, was specifically induced in the hypocotyl in response to BR treatment.

The antagonistic effects of BR and GA on *GA5* transcription may be explained via interaction(s) between upstream signaling components, or transcription factor(s) binding to common or distinct *GA5* promoter elements. Our data do not distinguish between these possibilities, although reporter analysis and RNA-blot analysis, performed either in the presence of ancymidol, which depletes endogenous GA levels, or in a double mutant impaired in GA and BR synthesis, show that the two hormones affect *GA5* transcription in a subtractive manner. More detailed promoter analysis is required to determine whether cis elements responsive to GA or BR are separable. To this end, we are introducing deletion derivatives of *GA5-LUC* into the *cpd* mutant.

Our current CHX experiments do not determine whether *GASA1* is a primary GA or BR response gene because the presence of translational inhibitors abolished its transcript levels. This does, however, suggest that labile factor(s) are required either for *GASA1* transcription or RNA stability. The effect of BR on *GASA1* expression appears similar to BR repression of transcription of the *CPD* steroid hydroxylase, whose expression also requires de novo protein synthesis (Mathur et al., 1998). We recently showed that GA induction of *GASA1* occurs at the transcriptional level using a *GASA1* promoter-*LUC* fusion (Raventos et al., 2000). However, it is unlikely that *GASA1* regulation by GA and BR are mediated through a common cis element because *GASA1* mRNA accumulation was repressed by BR, whereas BR failed to affect *GASA1-LUC* reporter activity in a transgenic *cpd* mutant line (data not shown). *GASA1* regulation by BR therefore may occur via a silencer located elsewhere in the *GASA1* gene, or at the post-transcriptional level. In a similar manner, post-transcriptional regulation of the *BRU1* gene by BR has been reported (Zurek and Clouse, 1994).

In contrast to *GASA1*, *GA5* mRNA levels were increased by CHX alone. In addition, mRNA levels seemed unaffected by GA or BR in the presence of CHX. This indicates that de novo protein synthesis is required for GA repression and BR induction of *GA5* transcription or mRNA accumulation. Regardless of whether *GA5* is a primary or late GA reponse gene, this is consistent with a simple model in which a labile repressor is required for GA negative feedback regulation of *GA5* (Meier et al., 2001).

Both *GA5-LUC* activity and endogenous *GA5* transcripts levels increased upon BR treatment, indicating that *GA5* is transcriptionally regulated by this hormone. However, *GA5* mRNA levels are only moderately increased by BR in the *cpd* mutant upon long-term EBR treatment, whereas *GA5-LUC* activity was induced up to 11-fold after 32 h (Fig. 5A). This suggests that *GA5* mRNA levels are regulated both at the transcriptional and post-transcriptional levels, whereas the *GA5-LUC* transgene lacks the corresponding post-transcriptional control. We have observed a similar result with new alleles of the late-flowering *fpa* mutant (Koornneef et al., 1991) in which the *GA5-LUC* reporter is highly expressed, whereas *GA5* transcripts levels are only slightly higher than in WT (Meier et al., 2001). In an alternate manner, it is possible that the *GA5-LUC* reporter used here lacks cis elements that negatively regulate *GA5* transcriptional induction by BR. In addition, BR regulation of both *GA5* and *GASA1* was clearly visible in *cpd*, whereas it was only moderate in WT (Fig. 4A). Moreover, the *GA5-LUC* reporter lacked apparent BR regulation in WT seedlings, whereas a strong BR up-regulation was detected in *cpd* (Fig. 5A). In a similar manner, BR induction of the xyloglucan endotransglycosylase homologs *MERI-5* and *TCH4* was only visible in BR-deficient mutants (Kauschmann et al., 1996). Two complementary explanations may account for these differences.

First, hormonal desensitization pathways necessary for homeostatic growth control may be less active in hormone deficient mutants than in WT. Second, tissue-specific hormonal effects may be masked in WT if both hormonal and other signals contribute to basal levels of target gene expression.

In conclusion, results presented here confirm and extend earlier reports of interactions between the important plant growth regulators GA and BR. Although certain of these interactions appear to be antagonistic, BR induction of *GA5* indicates that BR potentiates GA activity, at least in certain tissues. Elucidation of both transcriptional and posttranscriptional mechanisms will be required to understand how BR and GA regulate common targets involved in the control of cell elongation and plant growth.

MATERIALS AND METHODS

Plant Material and Hormone Treatments

The T_4 Arabidopsis ecotype Col-0 line carrying 400 bp of the *GA5* promoter driving the expression of the *FF-LUC* reporter (*GA5-LUC*) is described elsewhere (Meier et al., 2001). This line is homozygous for a single locus carrying the kanamycin resistance marker and exhibits sufficient LUC activity for bioluminescence imaging. The *GA5-LUC* reporter was introduced by crossing into the T-DNA-tagged *cpd* mutant (a gift from Csaba. Koncz, Max-Planck Institut fur Zuchtungsforschung, Koln, Germany), followed by segregation analysis for kanamycin resistance and *cpd* dwarfism in the F_3 population. Double mutants impaired in both GA and BR synthesis were generated by fertilizing the GA-treated *ga1-1* mutant (Koornneef and van der Veen, 1980) with *cpd* pollen. F_1 generation seedlings were selected for hygromycin resistance, which cosegregates with the *cpd* mutation. F_2 generation seedlings homozygous for *ga1-1* (i.e. required exogenous GA for flowering) and heterozygous for *cpd* (i.e. hygomycin resistant, not *cpd* phenotype) were amplified to produce the F_3 population, from which double mutants (25%) were visually selected. Joanne Chory (The Salk Institute for Biological Studies, La Jolla, CA) provided *bri1-1* seeds. Seeds were surface sterilized and germinated on Murashige and Skoog medium supplemented with 0.7% (w/v) agar and 1% (w/v) Suc. Unless specified, hormone treatments were performed on 2-week-old seedlings, which were transferred to liquid Murashige and Skoog medium 24 h prior to treatment. Exogenous application of GA (50 μM GA_3, Sigma-Aldrich, Vallenbæk, Strand, Denmark) and BR (0.1–1 μM 24-EBR, Sigma) was performed by adding the hormones to the medium for various times as indicated.

Generation of Arabidopsis Mutants

Approximately 25,000 bulked seeds were γ-irradiatiated (60 kRad) from a cobalt^{60} source (RISØ Industrial Irradiation, Risø, Denmark). M_1 seeds were bulked in pools of 1,250 seeds each and propagated in a long-day greenhouse. The *bri1-201* mutant was visually selected from M_2 progeny.

Reporter Assays

LUC bioluminescence imaging was performed as previously described (Raventos et al., 2000; Meier et al., 2001). In brief, bioluminescence was measured after spraying transgenic plants expressing either the *GA5-LUC* or the *GA4-LUC* reporters uniformly with 5 mM luciferin (JBL/Promega, Madison, WI) in 0.01% (w/v) Triton X-100. Plates were imaged three times for 5 min, and a bright-field reference image was taken thereafter. The first image was discarded due to chlorophyll phosphorescence, and the two remaining LUC images analyzed with the image-1/Metamorph system version 3.0 (Universal Imaging Corp., Downingtown, PA).

RNA Analysis

Total RNA was extracted from plant tissue using the RNAgent kit (Promega) and Poly-A RNAs isolated using the PolyA-tract kit IV (Promega). Poly-A RNA samples (1 μg) were size fractionated on 1.2% (w/v) agarose/formaldehyde gel and blotted onto Hybond N^+ membranes (Amersham, Pharmacia Biotech, Horsholm, Denmark). Transcript levels of genes involved in GA and BR biosynthesis (*GA5*, *GA4*, and *CPD*), genes encoding GA-signaling pathway components (*RGA* and *GAI*), as well as GA-regulated genes (*GASA-1*) were investigated. Ribonucleic ^{32}P-CTP antisense probes were synthesized using T7 RNA polymerase (Ribokit, Promega) from partial cDNA 3′ sequences cloned in the pGEM-Teasy vector (Promega) using the following primer combinations: GA5, aaggcctttgtggtcaatatcggc and gagatgctaaaaggtgttattgcc; GA4, ggtccgaaggtttcaccatcac and gagctttgttgaagtgagttgc; RGA, tggttcgtccggtttagcgccg and cagttcggtttaggtcttggtcc; GAI, cgggtctgctgggtttgcgg and tagtttggcttcggtcggaaatc; and GASA-1, ctctccttggagaatcatggct and acactcacaacgacaacgtacg. Hybridization and washing conditions were performed as recommended by the manufacturer. As a control, radioactive signals were quantified on all membranes by comparison to signals obtained after subsequent blotting with an elongation factor alpha probe (EF1-α, Axelos et al., 1989), whose transcript levels appeared unaffected by either GA_3 or EBR treatment (not shown).

Mapping of the Dwarf Mutant

Plants heterozygous for the *bri1-201* allele conferring dwarfism (Col-0 ecotype) were crossed to the Landsburg *erecta* ecotype. Mapping of the mutation was performed on F_2 progeny exhibiting the dwarf phenotype using simple sequence-length polymorphism markers (Bell and Ecker, 1994). Sequencing of the BRI gene from the bri1-201 mutant

was performed with an ABI prism sequencer (Perkin Elmer, Nærum, Denmark).

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