Brassinosteroids Interact with Auxin to Promote Lateral Root Development in Arabidopsis[1]

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Plant hormone brassinosteroids (BRs) and auxin exert some similar physiological effects likely through their functional interaction, but the mechanism for this interaction is unknown. In this study, we show that BRs are required for lateral root development in Arabidopsis and that BRs act synergistically with auxin to promte lateral root formation. BR perception is required for the transgenic expression of the β-glucuronidase gene fused to a synthetic auxin-inducible promoter (DR5::GUS) in root tips, while exogenous BR promotes DR5::GUS expression in the root tips and the stele region proximal to the root tip. BR induction of both lateral root formation and DR5::GUS expression is suppressed by the auxin transport inhibitor *N*-(1-naphthyl) phthalamic acid. Importantly, BRs promote acropetal auxin transport (from the base to the tip) in the root. Our observations indicate that BRs regulate auxin transport, providing a novel mechanism for hormonal interactions in plants and supporting the hypothesis that BRs promote lateral root development by increasing acropetal auxin transport.

Brassinosteroids (BRs) and auxin are two important phytohormones known to regulate many of the same aspects of plant growth and development, including cell division and expansion, vascular differentiation, root growth, and senescence. Several physiological studies show that some auxin-induced responses were synergistically enhanced by BR treatments (Clouse and Sasse, 1998). Strong synergism between BRs and auxin was reported in promoting cell expansion in the azuki bean (*Vigna angularis*), pea (*Pisum sativum*) epicotyl, and bean (*Phaseolus vulgaris*) hook bioassays (Yopp et al., 1981). BRs and indole-3-acetic acid (IAA) also showed synergistic interaction in inducing the bending of the leaf lamina of dwarf rice (*Oryza sativa*) seedlings and the inclination of rice lamina joint (Cao and Chen, 1995; Takeno and Pharis, 1982). Moreover, the BR-induced bending and inclination can be inhibited by high concentration of IAA transport inhibitor 2,3,5-triiodobenzoic acid (Cao and Chen, 1995; Takeno and Pharis, 1982). The ethylene production by etiolated mung bean hypocotyls is also synergistically promoted by BRs and auxin (Arteca et al., 1983). These physiological observations suggest a cross-talk between BRs and auxin. This interaction has been further supported by recent microarray analyses, which show that many early auxin responsive genes are also regulated by BRs (Goda et al., 2002; Müssig et al., 2002; Yin et al., 2002). On the other hand, auxin increases the BR responsive gene *TCH4* expression (Xu et al., 1995).

Based on the study of rice leaf lamina bending response, Takeno and Pharis speculated that this BR-induced response might be mediated via an increase in auxin levels (Takeno and Pharis, 1982). However, this notion has not been supported by studies in other plant species (Cohen and Meudt, 1983; Sasse, 1990). Cohen and Meudt (1983) reported that although BR enhanced the IAA-induced bending response of *Phaseolus vulgaris* internode, IAA uptake, metabolism, and transport were not affected by BR treatment directly. Sasse (1990) also proposed that the effect of BRs on promoting elongation of pea stem segment did not depend on auxin as a mediator because BR-induced elongation showed different characteristics to the auxin-induced growth. Therefore, the mechanisms for auxin-BR cross-talk remain mysterious.

In a previous study, we found that transgenic expression of constitutively active (CA-rop2) and dominant negative (DN-rop2) mutants of ROP2 GTPase in Arabidopsis induced many phenotypes related to auxin and BR actions and altered responses to both exogenous auxin and BRs (Li et al., 2001). For example, CA-rop2 expression increased shoot apical dominance, lateral root formation, hypocotyl elongation, and enhanced responses to both IAA and BR. These observations could be explained by one or more of the following three possibilities: (1) ROP signaling regulates both auxin and BR action independently, (2) ROP signaling regulates the action of either hormone whereas the two hormones interact, or (3) ROP signaling directly participates in a cross-talk between the two hormones. As a first step in distinguishing these

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possibilities, we sought to determine the functional interaction between BR and auxin and the underlying mechanism for this interaction.

In this report, we show that BR regulation of lateral root development is dependent upon auxin transport in Arabidopsis and that BRs increase acropetal auxin transport. These observations provide evidence that BR and auxin functionally interact at least in part through BR regulation of auxin transport.

RESULTS

BR Induction of Lateral Root Formation Is Suppressed by Auxin Transport Inhibitor

Low concentrations of BRs (≤0.1 nM) have been shown to promote root growth in Arabidopsis (Müssig et al., 2003), whereas higher levels of exogenous BRs inhibit root elongation in different root systems (Roddick and Guan, 1991; Sasse, 1994). However, reports on BR effect on adventitious and lateral root formation are very inconsistent (Yopp et al., 1981; Sathiyamoorthy and Nakamura, 1990). We found that the lateral root number decreased dramatically in the BR-insensitive mutant *bri1* and the application of brassinolide (BL) can partially rescue this defect in a weak *bri1* allele, but not in a strong *bri1* allele (data not shown).

To further assess the role of BRs in the regulation of lateral root formation, we performed a BR dose response of the primary root elongation and lateral root growth. As shown in Figure 1A, the number of lateral roots increased in response to 1 to100 nM BL with 10 nM being opitimal, inducing nearly an 8-fold increase in lateral root formation (Fig. 1A). Consistent with previous reports (Müssig et al., 2003), the elongation of primary roots was inhibited by 1 to 100 nM BL; the average root length of seedlings treated with 10 nM BL was only 50% of control (Fig. 1A). However, BL induction of lateral root formation was not quantitatively correlated with BL inhibition of primary root elongation. For example, the increase in lateral root number from 5 nM BL to 10 nM BL was 3.7-fold, but the change in primary root elongation within the same BR concentrations was only 1.3-fold. These results demonstrate that BL positively regulates the development of lateral roots.

It is known that auxin is crucial for lateral root development and lateral root emergence can be blocked by the auxin transport inhibitor *N*-(1-naphthyl) phthalamic acid (NPA; Reed et al., 1998; Casimiro et al., 2001). Under our growth condition, we found that 2 μM NPA not only inhibited lateral root formation in wild-type seedlings but also reduced BL promotion of lateral root formation (Fig. 1B). These results imply a potential interaction be-

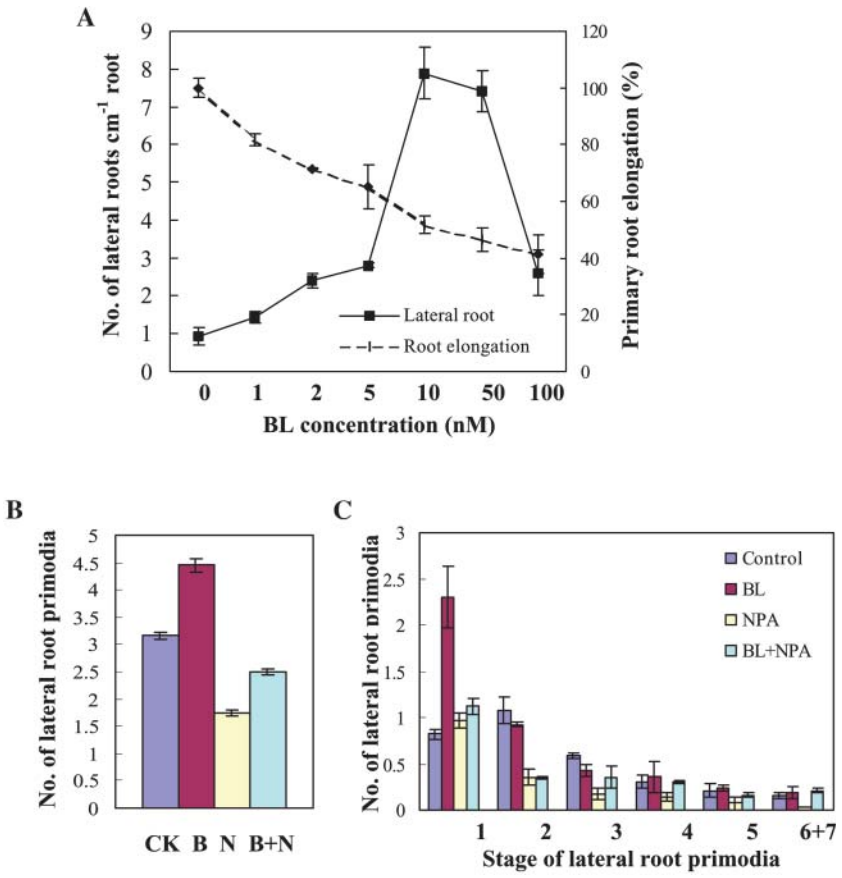

Figure 1. Brassinosteroids promote lateral root formation A, Low concentration of BL promotes lateral root formation. Col-0 seedlings were grown vertically for 8 d on 1/2 MS plates containing 0, 1, 2, 5, 10, 50, and 100 nM BL, respectively. The number of lateral roots and visible lateral root primodia per centimeter of primary root were counted. Values in the figure represent the means ± SE of four replicates. B, BL promotion of lateral root primodium development is suppressed by NPA. Five-day-old *DR5*::*GUS* seedlings grown vertically on 1/2 MS plates were transferred to plates containing 50 nM BL and/or 2 μM NPA for another 2 d. After histochemical GUS staining, the number of LRPs was counted under microscope. CK, control; B, BL; N, NPA; B + N, BL plus NPA. Values in the figures represent the means ± SE of three replicates. C, BL promotes the development of the early stages of lateral root primordia. Different stages of lateral root primordia (stages 1–7) were determined according to Malamy and Benfey (1997).

tween BRs and auxin in the promotion of lateral root formation, i.e. BRs may act through auxin to activate lateral root development.

Auxin is required for both initiation and emergence stages of lateral root formation (Casimiro et al., 2001; Bhalerao et al., 2002; Benková et al., 2003). To assess which stage of lateral root formation is affected by BR, we quantified the number of lateral roots or lateral root primodia (LRP) of different stages after BR and/or NPA treatments. We used GUS expression pattern in DR5::GUS transgenic seedlings to facilitate the examination of different stages of LRP, as DR5::GUS is expressed at all stages of LRP (Benková et al., 2003) and thus facilitates the identification of early LRP. Two days following BL and/or NPA treatments, roots were subject to GUS staining. The LRP stages were determined by the number of cell layers expressing DR5::GUS, as described previously (Malamy and Benfey, 1997). Fifty nM BL increased the number of stage 1 LRP by about 3-fold, but did not change the number of stage 2 to stage 7 LRP (Fig. 1C), suggesting that BRs modulate the initiation of LRP. Consistent with an earlier study using 1 μM NPA (Casimiro et al., 2001), we found that 2 μM NPA dramatically decreased the number of stage 2 to 7 LRP but slightly increased the number of stage 1 LRP (Fig. 1C). Interestingly, NPA almost completely suppressed BL induced increase in the number of stage 1 LRP (Fig. 1C). These results are consistent with the hypothesis that BRs act through auxin in promoting LRP initiation.

Interestingly, BL treatment rescued NPA-inhibition of LRP at the later stages after stage 2 (Fig. 1C). These results suggest BRs and auxin may interact differently in the regulation of early stages of LRPs from the later stages, consistent with the existence of multiple distinct mechanisms involved in the development of different stages, as shown by differential spatiotemporal expression of different PINs during the development of LRPs (Benková et al., 2003).

Synergistic Effects of BL and IAA on Lateral Root Formation

If BRs and auxin interact in the regulation of lateral root development, we would expect BRs and IAA to act synergistically. In the absence of BL, up to 20 nM IAA did not cause significant effects on lateral root formation, while 50 nM IAA dramatically increased the number of lateral roots (Fig. 2). Treatment with 1 nM BL alone increased the number of lateral roots per centimeter primary root by 1.6-fold (Figs. 1A and 2). The relative density of lateral roots in treatments with a combination of 1 nM BL and 1, 5, or 20 nM IAA were 2.64-, 2.07-, and 1.95-fold of that in treatment with 1, 5, and 20 nM IAA alone, respectively (Fig. 2). These results indicate that at low levels of auxin, BL and auxin have a synergistic effect on the promotion of lateral root formation. When IAA concentration increased to 50 nM, however, no synergistic effects were seen with BL (Fig. 2). One possible explanation of these

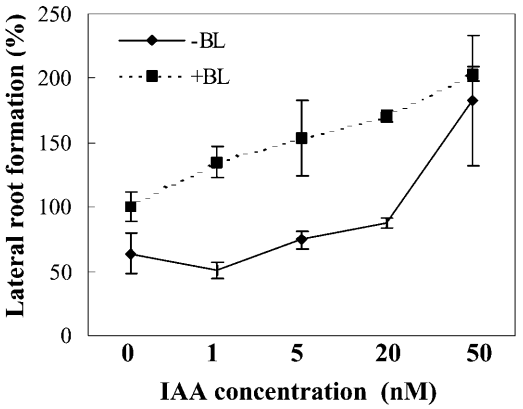

Figure 2. BL and IAA act synergistically to promote lateral root development. Col-0 seedlings were grown vertically on 1/2 MS plates containing 0, 1, 5, 20, and 50 nM IAA with or without 1 nM BL, respectively, for 8 d. The number of lateral roots and visible lateral root primodia per centimeter of the primary root was counted. Data are represented as the ratio of the number of lateral root within unit length in each treatment relative to the number of lateral root in 1 nM BL treatment. Values in the figures represent the means ± SE of three replicates.

results is that BL increases either auxin levels or cell sensitivity to auxin in roots. When the exogenous auxin levels were low, the application of BL could increase the endogenous auxin level, transport, or sensitivity and promote the initiation of lateral roots. In the presence of high levels (>20 nM) of exogenous auxin, signals activating lateral root initiation may be saturated, masking the synergistic effect of auxin and BL.

Brassinolide Enhanced the Expression of Auxin-Inducible Promoter in a Tissue-Specific Manner

To probe the mechanism by which BL interacts with auxin, we examined the effect of BRs on the expression of DR5::GUS. The synthetic promoter DR5 is sensitive to auxin in a dosage dependent manner (Ulmasov et al., 1997), and its activity is thought to reflect endogenous auxin levels in some tissues, especially in roots (Sabatini et al., 1999; Casimiro et al., 2001; Benková et al., 2003). In the control (Fig. 3A), expression of DR5::GUS is mainly localized to the tip, serrate initiation site and developing vasculature of young leaves, the edge of cotyledons and root tips (including the root meristem region and the columella), as reported previously (Sabatini et al., 1999).

Treament with 50 nM BL enhanced GUS expression in the tip of cotyledons and leaves and caused GUS expression throughout their margins. A similar observation was made in a recent study (Nakamura et al., 2003). In roots, BL treatments enhanced GUS expression in the root apex, and the most pronounced changes occurred in the stele (Fig. 3A). In the control, GUS expression in the stele is weak, exhibiting a

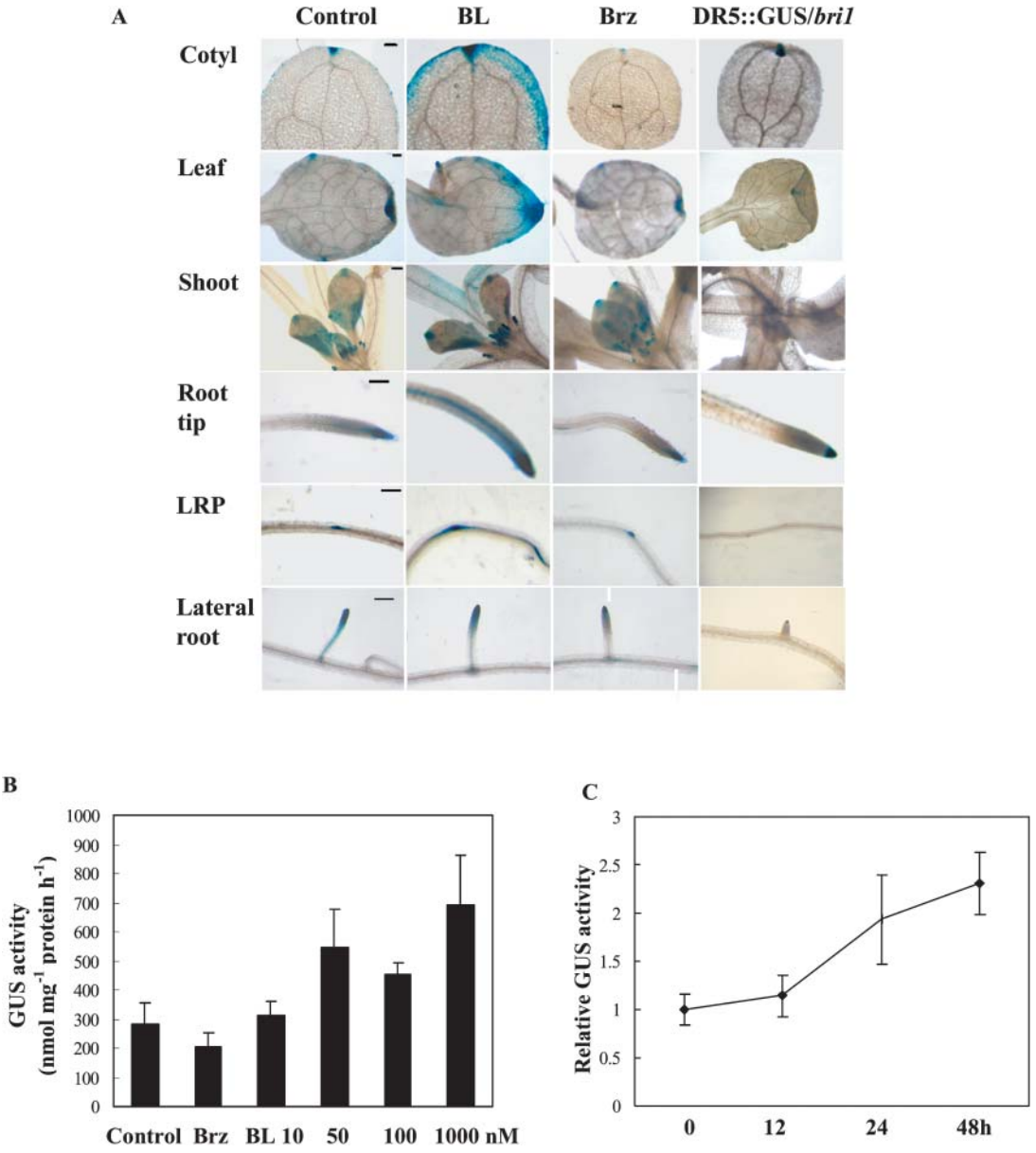

Figure 3. Brassinosteroids induce DR5::GUS expression. A, DR5::GUS expression in WT seedlings treated with BL or Brz or in the *bri1*-119 mutant. Four-day-old *DR5*::*GUS* seedlings grown in liquid medium were treated for 48 h with 50 nM BL or 1 μM Brz, respectively. *DR5*::*GUS*/ *bri1* seedlings were grown in liquid medium for 6 d before histochemical GUS staining was performed. Images show different parts of seedlings: cotyledons (Cotyl), first true leaves, shoots, root tips, LRP and mature lateral root. Scale bar = 100 μm. B, BL dose response of BL-induced DR5::GUS expression. Four-day old liquid-cultured *DR5*::*GUS* seedlings were treated with 1 μM Brz, or 0, 10, 50, 100, or 1,000 nM BL, respectively for 24 h. Whole seedlings were used for the assay. Values in the figure represent the means ± SE of three replicates. C, Time-course analysis of BL effect on DR5::GUS expression. Four-day-old *DR5*::*GUS* seedlings were treated with 50 nM BL or dimethyl sulfoxide mock treatment for 0, 12, 24, and 48 h, respectively. Whole seedlings were used for the assay. Data are represented as a ratio of GUS activity per microgram protein from the BL treated samples relative to the mock-treated samples. Values in the figure represent the means ± SE of three replicates.

tip-high gradient with no detectable GUS staining in the elongation zone. In contrast, in BL-treated roots, GUS staining in the stele was much stronger and extended to the elongation region. In the control DR5::GUS is strongly expressed at all stages of lateral root primodia. BL treatments extended GUS staining to the flanking region of LRPs (Fig. 3A). Treatments with 10 nM BL were shown to enhance DR5::GUS expression in root tips but not the stele (Nakamura et al., 2003).

To investigate whether the DR5::GUS expression was regulated by endogenous BRs, we examined the effect of mutations in *BRI1* and treatments with brassinozale (Brz), an BR biosynthesis inhibitor (Asami and

Yoshida, 1999). In *bri1-119* background, the GUS staining in cotyledons, first true leaves, shoots, and lateral roots was considerably decreased compared to that of the control (Fig. 3A). Brz treatment also caused some decrease in GUS activity in cotyledons, young leaves, and lateral roots (Fig. 3A).

To confirm the DR5::GUS histochemical observation, enzymatic assay of GUS expression was performed. Consistent with the histochemical analysis, quantitative assay showed that Brz reduced DR5::GUS expression by 30% (Fig. 3B) at the whole seedling level. In contrast, BL increased GUS relative activity in a concentration-dependent manner with slight increase (11%) by 10 nM BL and significant induction (140%) by 1,000 nM BL (Fig. 3B). Kinetic studies showed that the DR5::GUS expression was induced within 24 h (93% increase compared to mock treatment) and further increased at 48 h (130% increase; Fig. 3C). Thus, quantitative GUS activity analysis suggests BL causes an overall increase of DR5::GUS expression, which is consistent with the GUS histochemical analysis. These results are also consistent with previous reports indicating that BRs induce the expression of several classes of auxin-inducible genes (Goda et al., 2002; Müssig et al., 2002; Yin et al., 2002; Nakamura et al., 2003).

BR Did Not Increase Free IAA Levels

To assess whether the induction of DR5::GUS expression is due to an overall increase in auxin accumulation, we measured free IAA levels in *bri1-119* seedlings and in wild-type seedlings treated with 50 nM BL or 1 μM Brz for 24 h, respectively. The results showed that the *bri1* mutant had a normal IAA level and Brz had no effect on IAA levels (Fig. 4). BL treatment actually decreased IAA content (Fig. 4), consistent with the observation that IAA levels increased in the BR-deficient *det2* mutant (Nakamura et al., 2003). These results indicate that the induction of DR5 expression by BL is not through the increase of auxin level in the whole plant.

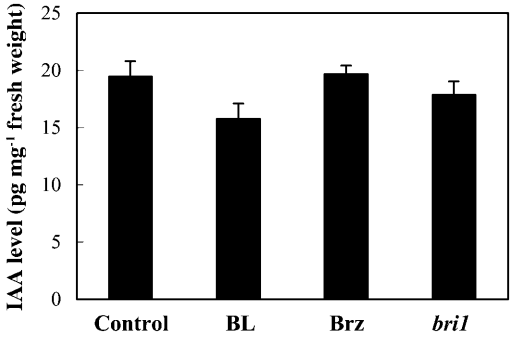

Figure 4. Effects of BRs on free IAA levels. The *bri1-119* and wild-type Col-0 seedlings were grown in liquid medium for 7 d. The wild type seedlings were treated with 50 nM BL or 1 μM Brz, respectively, for 24 h before IAA extraction. Ten milligrams of whole seedlings were used for the extraction. Values in the figure represent the means ± SE of three replicates.

BR Treatment Promotes Auxin Acropetal Transport in Roots

Since BL did not affect the overall IAA level of whole seedlings, we assessed whether or not BRs regulate auxin transport. In seedlings treated with NPA alone, GUS staining increased in the margin of cotyledons and leaves (Fig. 5). When seedlings were treated with the combination of NPA and BL, GUS expression in the margin of cotyledons and young leaves became much stronger, compared to either NPA or BL treatments alone (Fig. 5). The most likely interpretation of this result is that auxin is produced in and transported from the leaf margin, where BL either increases auxin synthesis or sensitivity.

In roots, NPA treatments slightly enhanced GUS expression in the apex but abolished the tip-high gradient of GUS expression along the stele. This observation is thought to be due to the NPA inhibition of both auxin acropetal and basipetal transports (Reed et al., 1998; Casimiro et al., 2001). Interestingly, NPA completely blocked BR induction of GUS expression in root stele. GUS staining in root tips was slightly stronger in BL/NPA treatment than NPA treatment (Fig. 5). Therefore, these results are consistent with a role for BRs in promoting auxin acropetal transport in the root.

We next determined whether BRs indeed affect auxin acropetal transport. As shown in Figure 6, low concentrations of BL (1 and 5 nM) seemed to slightly increase acropetal transport, but 25 nM BL dramatically promoted auxin acropetal transport by more than 200% (Fig. 6). Thus, the effective BL concentration for promoting acropetal auxin transport is similar to those promoting lateral root initiation and DR5::GUS expression in roots.

DISCUSSION

Functional interactions beween different phytohormones are an important aspect of hormone action in plants, but the mechanisms behind hormonal cross-talks are poorly understood. In this study, we have demonstrated that BRs interact with auxin by affecting auxin polar transport. This novel mechanism for hormonal cross-talk explains our finding that BRs promote the initiation of lateral root primordia in an auxin transport dependent manner.

Our work has provided convincing evidence for a functional interaction of BRs with auxin in Arabidopsis, although several previous studies have implicated this interaction in other species (see introduction). We have shown that BRs promote the initiation of lateral root primordia, a process mediated by auxin, and that this BR action is suppressed by NPA, an auxin transport inhibitor. Furthermore, BRs act synergistically with auxin in the regulation of this

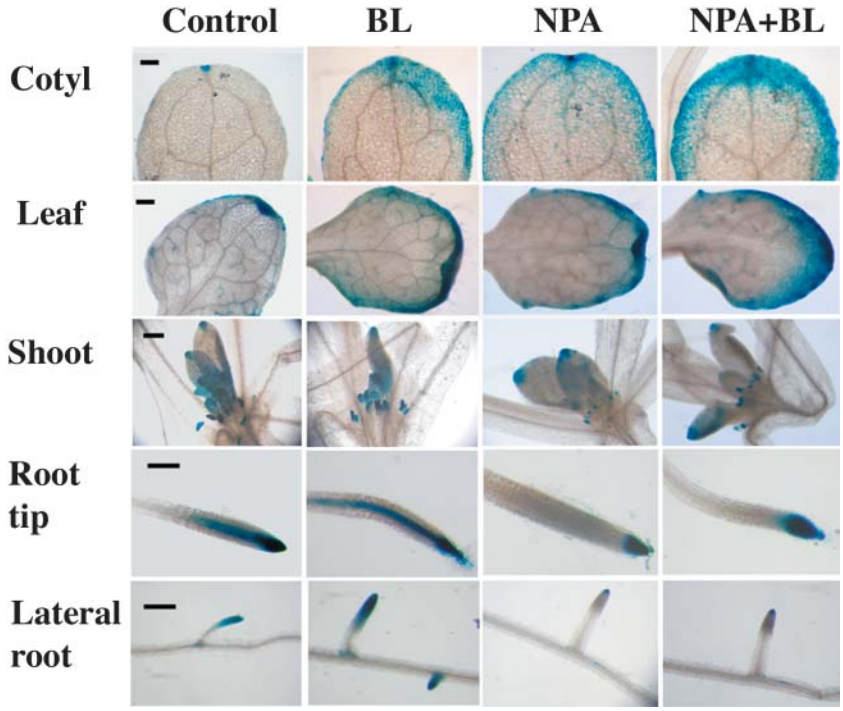

Figure 5. Effects of NPA on BR induction of DR5::GUS expression. *DR5::GUS* seedlings were grown in liquid medium for 4 d and were treated with dimethyl sulfoxide (mock treatment), 50 nM BL, 2 μM NPA, or 50 nM BL plus 2 μM NPA for 48 h before histochemical GUS staining as shown in Figure 3. Scale bar =100 μm.

process. We found that BR signaling is crucial for BR regulation of lateral root development, as mutations in *BRI1* affect this process (data not shown).

The BR interaction with auxin in the regulation of lateral root development is likely a universal phenomenon in various plant species. Low concentrations of BRs have been shown to promote the formation of adventitious roots in excised soybean (*Glycine max*) hypocotyls (Sathiyamoorthy and Nakamura, 1990). The pea *lk* mutant, which is deficient in BR accumulation, has reduced number of lateral roots (Yokota et al., 1997). However, the *LK* gene has not been cloned, so it is not clear whether it is a BR biosynthesis gene.

Several lines of evidence show that BRs modulate auxin polar transport. BRs enhance the expression of DR5::GUS along the stele of the primary roots near the root tip. Conversely, the DR5::GUS expression in the root tip is dramatically reduced in the *bri1-119* mutant. Importantly, the BR induced change in the GUS expression pattern is suppressed by NPA. It is known that auxin produced in the shoot is transported through the stele to the root tip and redistributed to other parts of the root by basipetal transport through the outer layers of root cells (Lomax et al., 1995; Jones, 1998). The BR enhanced GUS expression in the stele can be explained by an excessive accumulation of auxin resulting from enhanced acropetal auxin transport and reduced basipetal transport. We have shown that acropetal auxin transport was drastically enhanced by BRs at the concentration similar to the one effective in inducing DR5::GUS expression. Preliminary results suggest that BRs also weakly inhibit basipetal auxin transport (Brady and Muday, unpublished data). Since acropetal auxin polar transport in the root has been shown to be critical for lateral root development (Reed et al., 1998), we propose that BRs promote lateral root development at least in part by increasing acropetal auxin transport.

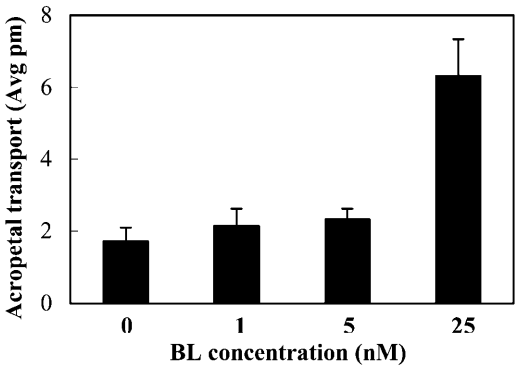

Figure 6. BR promotes auxin acropetal transport in the root. Wild-type Col-0 seedlings were grown vertically on 1/2 MS soild medium containing 0, 1, 5, or 25 nM BL for 7 d and the roots were used for transport assay as described in text. The unit in the *y* axis Avg pm indicates average pmoles of IAA moved as described in text.

In the process of lateral root formation, both the initiation and emergence of lateral root primordia require auxin (Casimiro et al., 2001; Bhalero et al., 2002; Benková et al., 2003). Our results indicate that BRs are also important for the initiation of lateral root primordia (Fig. 1C). BR induction of this process is dependent upon auxin transport, consistent with BR promotion of acropetal auxin transport. Casimiro et al. (2001) show that 10-d-old *shoot meristemless 1 (stm1)* mutant seedlings have similar numbers of lateral roots as wild-type seedlings. On the basis of this

observation, they propose that basipetal but not acropetal transport of auxin in roots is required for the initiation of lateral roots. However, this interpretation is difficult to reconcile with the report that acropetal auxin transport is required for the development of lateral root (Reed et al., 1998). We have shown that NPA treatments strongly induced DR5::GUS expression in the margin of cotyledons, which exhibited much weaker GUS expression in untreated seedlings, indicating that auxin is produced in and rapidly transported away from the cotyledon margin (Fig. 5). This auxin most likely provides an alternative source for acropetal transport in roots to the apex, which could explain why *stm1* seedlings exhibit normal lateral root development.

How could regulation of acropetal auxin transport be important for the initation of lateral roots? Since acropetal transport takes place in the stele, auxin in this pathway could be readily transported laterally to the pericyle cell to induce the initiation of lateral root primordia there (Reed et al., 1998; Benková et al., 2003). Alternatively, the increased accumulation of auxin in the root tip due to BR-enhanced acropetal transport could provide a greater auxin source for basipetal auxin transport. Our result showing that exogenous auxin in the medium was unable to promote lateral root formation in a BR-deficient *det2* mutant to the same level as in wild-type seedlings (data not shown), tends to support the first explanation. Several recent studies indicate that the local accumulation and redistribution of auxin is crucial during plant organ formation (Benková et al., 2003; Friml et al., 2003; Reinhardt et al., 2003). The site of primodium initiation is determined by local auxin accumulation, while the apical-basal auxin gradient is established in the primodium by PIN-mediated auxin transport (Benková et al., 2003; Friml et al., 2003; Reinhardt et al., 2003). It would be interesting to see if BRs regulate auxin transport through the modulation of PIN protein accumulation and/or localization.

Many questions remain about the mechanisms by which BRs interact with auxin. An important question is whether BR regulation of auxin transport is a general mechanism for BR-auxin interaction, which likely regulates several other aspects of plant growth and development apart from lateral root initiation (see introduction). In addition, our results do not rule out other possible mechanisms for BR interaction with auxin. Our whole seedling assays of IAA levels show BRs do not increase overall IAA levels, but did not exclude a localized and subtler modulation of IAA levels by BRs. In fact, BL induction of DR5::GUS expression in the cotyledon and leaf margins was enhanced by NPA treatments, suggesting a potential role for BRs in up-regulating auxin levels or sensitivity in this tissue. This potential BR induction of IAA production could also contribute to BR promotion of lateral root development. It was shown that BL did not induce the expression of early auxin inducible genes until 1 h after BL treatments, whereas auxin induction of the expression of these genes occurs within 30 min (Goda et al., 2002). The difference between the kinetics of BR and auxin induction of auxin-responsive genes would be more difficult to explain on the basis of BR action thorugh auxin transport. However, it could be explained by BR regulation of auxin levels or by BR signaling-dependent production of a factor that enhances auxin sensitivity. Furthermore, it remains possible that BR-auxin cross-talk involves auxin action through its regulation of BR accumulation or sensitivity. Nonetheless, our work provides an important starting point for future dissection of what appears to be complex mechanisms of BR-auxin cross-talks.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

The Arabidopsis Columbia ecotype (Col-0), BR insensitive mutant *bri1-119* in Col-0 background (Li and Chory, 1997), *DR5::GUS* transgenic plants, and *DR5::GUS/bri1-119* were used for this study. For root growth analysis, seeds were surface sterilized with 70% ethanol for 2 min and 15% bleach for 15 min and washed three times with sterile water. Sterile seeds were plated on an agar medium [1/2 Murashige and Skoog medium (MS) plus 1% (w/v) Suc and 0.8% (w/v) phytagar (Gibco BRL, Gaithersburg MD)]. Seedlings were grown at 22°C with a light regime of 8 h darkness and 16 h light (33 μmol m^{-2} s^{-1}).

Measurement of the Growth of Arabidopsis Primary Roots

Seedlings were grown vertically on 1/2 MS plate for 5 d after germination and then transferred to 1/2 MS medium containing different concentration of BL, and/or IAA for another 3 d. Seedlings were photographed using a Nikon (COOLPIX 950, Tokyo) digital camera, and the length of primary roots was measured using the MetaMorph version 4.5 software (Universal Imaging, West Chester, PA). The number of lateral roots and lateral root primodia were counted under light microscope (Nikon Labophot-2) after fixed in 70% (v/v) ethanol.

Histochemical and Quantitative GUS Assays

Histochemical assays for GUS activity were performed as described by Jefferson et al. (1987) with minor modification. Five day-after-germination DR5::GUS or DR5::GUS/*bri1* seedlings were treated with BL or inhibitors and then submerged in GUS staining buffer containing 1 mM 5-bromo-4-chloro-3-indolyl β-D-GlcUA, 100 mM sodium phosphate (pH 7.5), 0.5 mM potassium ferricyanide, 0.5 mM possium ferrocyanide, 10 mM EDTA, and 0.1% (v/v) Triton X-100. Tissues were incubated at 37°C for 3 h and chlorophyll in green tissues was cleared by 70% (v/v) ethanol. Images were taken under a dissecting microscope (Nikon SMZ800).

Quantitative GUS assays were performed according to Gallagher (1992). 100 mg whole seedlings were ground in 300 μL extraction buffer (50 mM $NaPO_4$, pH 7.0, 10 mM EDTA, 10 mM β-mercaptoethanol, 0.1% Triton X-100, and 0.1% [w/v] sarcosyl) in a microcentrifuge tube. Cell debris was removed from the homogenates by centrifugation at 12,000 rpm for 15 min. 20 μL of the homogenates were mixed with 400 μL GUS assay buffer (2 mM 4-methylumbelliferyl β-D-glucuronide in extraction buffer) and incubated at 37°C for 1 h. Aliquots of 100 μL were taken and reaction was stopped by adding 1.9 mL stop buffer (0.2 M Na_2CO_3). Fluorescent products were quantified with a fluorometer (SPEX Fluorology, Edison, NJ).

Measurement of Auxin Transport

Arabidopsis wild-type Col-0 seedlings were grown vertically on 1/2 MS plates with or without BL. Acropetal auxin transport was measured in 7-d-old vertically grown seedlings as modified from Reed et al. (1998). Three milliliter mixtures containing 1% (w/v) agar and 50 nM ^{3}H-IAA (26 Ci $mmol^{-1}$,

Arlington Heights, IL) were prepared in 4-mL scintillation vials and allowed to harden. This cylinder containing ^{3}H-IAA agar was placed on the root just below the root/shoot junction. Plates remained oriented vertically in the dark to avoid IAA degradation. IAA transport was measured after 18 h by excising a 5-mm segment of the root tip, placing it into 2.5 mL of scintillation fluid, and the amount of radioactivity within each sample was determined using a Beckman LS6500 scintillation counter for 2 min.

Measurement of Auxin Level

Seven-day-old *bri1-119* and Col-0 wild-type seedlings were used. The wild-type seedlings were treated with 50 nM BL or 1 μM Brz for 24 h on the sixth day. Ten milligrams of whole seedlings were used for IAA extraction. The extraction of IAA and GC-MS were performed as described (Edlund et al., 1995).

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