INTRODUCTION

Plants must adapt their form to survive a complex and changing environment. The extensive molecular interplay between external (i.e., light and temperature) and internal (i.e., circadian clock) signals allows for a high degree of developmental plasticity. Light-directed seedling growth (photomorphogenesis) is one of the best-characterized examples of a highly dense regulatory network. Small molecule hormones are critical for relaying information about the light environment, as well as a diverse set of additional metabolic, environmental, and developmental cues (Vaishak et al., 2019; de Wit, Galvão, & Fankhauser, 2016). Hormones like auxin and brassinosteroids (BRs) play a central role in coordinating growth during photomorphogenesis.

Auxin promotion of seedling growth via ARF5 is dependent on the brassinosteroid-regulated transcription factors BES1 and BEH4

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Abstract

Seedlings must continually calibrate their growth in response to the environment. Auxin and brassinosteroids (BRs) are plant hormones that work together to control growth responses during photomorphogenesis. We used our previous analysis of promoter architecture in an auxin and BR target gene to guide our investigation into the broader molecular bases and biological relevance of transcriptional co-regulation by these hormones. We found that the auxin-regulated transcription factor Auxin Responsive Factor 5 (ARF5) and the brassinosteroid-regulated transcription factor BRI1-EMS-Suppressor 1/Brassinazole Resistant 2 (BES1) co-regulated a subset of growth-promoting genes via conserved bipartite cis-regulatory elements. Moreover, ARF5 binding to DNA could be enriched by increasing BES1 levels. The evolutionary loss of bipartite elements in promoters results in loss of hormone responsiveness. We also identified another member of the BES1/BZR1 family called BEH4 that acts partially redundantly with BES1 to regulate seedling growth. Double mutant analysis showed that BEH4 and not BZR1 were required alongside BES1 for normal auxin response during early seedling development. We propose that an ARF5-BES1/BEH4 transcriptional module acts to promote growth via modulation of a diverse set of growth-associated genes.

KEYWORDS
Arabidopsis thaliana, auxin, brassinosteroids, growth related genes, promoter architecture, seedling development, transcriptional modules
Plants with defective responses to auxin or BRs show an array of phenotypes of light-grown plants even when grown in the dark (Arsenovsk, Galstyan, Guseman, & Nemhauser, 2012). The signaling pathways downstream of auxin and BRs are distinct. Auxin binds to the Transport Inhibitor Response 1/Auxin Signaling F-Box 1–5 (TIR1/AFB) family of F-box receptors and triggers the ubiquitination and degradation of Auxin/Indole-3-Acetic Acid (Aux/IAA) co-repressors. Loss of the Aux/IAAAS activates Auxin Response Factors (ARFs) to regulate gene expression (Chapman & Estelle, 2009; Luo, Zhou, & Zhang, 2018; Wang & Estelle, 2014). ARFs bind to an auxin response element (AuxRE, TGTCTC) and related cis-elements in target promoters (Boer et al., 2014; Chandler, 2016; Galli et al., 2018; Guilfoyle & Hagen, 2007; Stigliani et al., 2018). In contrast, BRs bind and activate the brassinosteroid-insensitive 1 (BRI1)-associated receptor complex at the plasma membrane. A phospho-relay cascade culminates in dephosphorylated and nuclear-localized transcription factors, including BRI1-EMS-Suppressor 1/Brassinazole Resistant 2 (Zhang, 2018; Wang & Estelle, 2014). In contrast, BRs bind and activate the Brassinosteroid-Insensitive 1 (BRI1)-associated receptor complex at the plasma membrane. A phospho-relay cascade culminates in dephosphorylated and nuclear-localized transcription factors, including BRI1-EMS-Suppressor 1/Brassinazole Resistant 2 (BES1/BZR2, hereafter BES1) and BZR1 (Li & Deng, 2005; Yin et al., 2005, 2002). BES1 and BZR1 regulate gene expression by binding to cis-elements (BES1/BZR2, hereafter BES1) and BZR1 (Li & Deng, 2005; Yin et al., 2005, 2002). BES1 and BZR1 regulate gene expression by binding to both cis-elements (CANNTG) and BBRE (CGTGG(T/C)G) cis-elements in target promoters (Sun et al., 2010; Yu et al., 2011).

Genetic, physiological, and genomic analyses demonstrate molecular and physiological responses of auxin and BRs are interdependent. BRs promote auxin transport, hence altering overall auxin distribution within the plant (Chaawanon & Wang, 2015; Li, 2005). BRs also regulate expression of genes involved in the core auxin response (Hardtke, 2007; Nakamura et al., 2006; Nemhauser, Mockler, & Chory, 2004), and a BR-regulated kinase targets members of the ARF family (Cho et al., 2013; Han et al., 2018; Hu et al., 2018; Vert, Walcher, Chory, & Nemhauser, 2008). Auxin stimulates de novo BR biosynthesis by directly regulating expression of DWARF4 (DWF4), a BR biosynthetic enzyme (Chung et al., 2011; Yoshimitsu et al., 2011). BRI1 is a direct target of activator ARF5/MONOPTEROS (hereafter, ARF5) (Sakamoto, Morinaka, Inukai, Kitano, & Fujioka, 2013). In addition, ARF6 and ARF7 were shown to interact with BZR1 to regulate shared target genes (Oh et al., 2014). Previously, we demonstrated that a bipartite element in the promoter of SAUR15 gene that includes a type of E-box called a HUB element (CACATG) and a variant of the AuxRE (TGTCTC) are bound by ARF5 and BES1, and that binding by both transcription factors is required for induction of expression by either hormone (Walcher & Nemhauser, 2012). In this work, we expanded this study to include other growth-associated genes with predicted bipartite elements in their promoters. We found that BES1 sensitizes hypocotyl response to auxin by enhancing ARF5 binding to shared target promoters. The evolutionary loss of the conserved promoter architecture with bipartite elements results in loss of hormone responsiveness. BEH4, a previously uncharacterized paralog of BES1, was found to act redundantly with BES1 as a major regulator of seedling growth. We propose a model where shared promoter architecture facilitates a coordinated and highly responsive growth controlling module encompassing genes from diverse families.

2 MATERIALS AND METHODS

2.1 Plant materials and growth conditions

The wild type is Arabidopsis thaliana ecotype Col-0 except beh1-2 and beh2-1 that are in Col-3 background. bes1-D (Yin et al., 2002), bzh1-D (Wang et al., 2002), bin2-D (He, Gendron, Yang, Li, & Wang, 2002), lng1-3 (Lee et al., 2006), xth17 (Sadidharan, Keuskamp, Kooke, Voesenek, & Pierik, 2014), pi(7-2 (Leivar et al., 2008), ARF5, ARF6, and ARF7 (Scherer et al., 2010), BES1, BES1:GFP (Yin et al., 2002), XTH19:GUS, and XTH19 (SAIL_76_B06, Col-3 background), beh3-1 (SALK_017577), and beh4-1 (SAIL_750_F08) and double mutants: beh1-2beh4-1 and beh1-2beh1-2 are described lines (Lachowiec, Mason, Schultz, & Queitsch, 2018). For detailed information on genotyping methods, primers, and generation of double mutants, see Data S1.

For seed production and crosses, plants were grown in a growth chamber under LD conditions. Seeds were surface sterilized (20 min in 70% ethanol, 0.01% Triton X-100, followed by a rinse in 95% ethanol) for all the physiological and molecular analyses. For hypocotyl and GUS assays, sterilized seeds were suspended in water and sown individually on plates containing 0.5× Linsmaier and Skoog (LS) (LSP03, Caisson Laboratories Inc, http://www.caissonlabs.com/) with 0.8% phytoagar (40100072-1, Plant Media: bioWorld, http://www.plantmedia.com/), and stratified in the dark at 4°C for 3 days. Plates were placed vertically in a Percival E-30B growth chamber set at 20°C in 30 µmol m−2 s−1 of photosynthetically active radiation white light with short-day (SD) conditions (8-hr light, 16-hr dark). For gene expression and ChIP assays, sterilized seeds were suspended in 0.1% agar (BP1423, Fisher Scientific, http://www.fisher.co.uk/), with 0.8% phytoagar (40100072-1, Plant Media: bioWorld, http://www.plantmedia.com/), and stratified in the dark at 4°C for 3 days, and grown horizontally as described above.

2.2 Chemical treatments

To prepare stock solutions, brassinosteroid (brassinolide (hereafter BL), 101, ChemiCon Inc. www.chemicon.com), IAA (705490, PlantMedia.com), and picloram (P5575, Sigma) were dissolved in 80% ethanol (50 mM for IAA, 1 mM for BL, 5 mM for PIC) as working stocks which were used to dilute directly into plate medium or treatment solution to the final concentration of 50 µM for IAA, 1 µM/0, 5 µM for BL, and 5 µM for PIC, respectively. Stock solutions were kept at −20°C until use.

2.3 Accession numbers

Sequence data from this article can be found in the Arabidopsis Genome Initiative or GenBank/EMBL databases under the following accession numbers: BES1 (At1g19350), BZR1 (At1g75080), BEH1 (At3g50750), BEH2 (At4g36780), BEH3 (At4g18890), BEH4 (At1g78700), ARF5 (At1g19850), IAA6 (At1g52830), XTH1
GUS staining

Seven-day-old (D8) seedlings (BES1\textsubscript{PRO}:BES1:GFP, ARF5\textsubscript{PRO}:ARF5: GFP and bes1-D × ARF5\textsubscript{PRO}:ARF5:GFP) were treated for 3 hr with 80% ethanol (mock) or 50 µM IAA in liquid 0.5× LS media on plate and crosslinked in 1% formaldehyde under vacuum on ice. Cross-linking was stopped by infiltrating in 0.125 M room temperature glycine solution. Seedlings were subsequently frozen in liquid nitrogen and ground to a fine powder with mortar and pestle. Samples were resuspended in nuclei extraction buffer (0.25 M Suc, 100 mM MOPS, pH 7.6, 10 mM MgCl\textsubscript{2}, 5% Dextran T-40, 2.5% Ficoll, 20 mM b-mercaptoethanol, and mini-Complete Protease Inhibitor tablet [04693124001; Roche Applied Science], filtered through Miracloth (475855; Calbiochem), and centrifuged to collect nuclei. Nuclei were lysed with nuclei lysis buffer (50 mM Tris–HCl, pH 8, 10 mM EDTA, and 1% SDS). ChiP dilution buffer was added (1.1% Triton X-100, 1.2 mM EDTA, 16.7 mM Tris, pH 8.0, 167 mM NaCl, and 0.01% SDS), and chromatin was fragmented using Bioruptor sonicator (Fisher Scientific, Bioruptor\textsuperscript{®} UCD-200). An aliquot of fragmented chromatin served as an input control for qPCR analysis, and the remainder was subjected to immunoprecipitation. Dynabeads protein A (100-02D; Invitrogen) coupled with anti-GFP (Ab290; Abcam) antibody was used to enrich for ARF5\textsubscript{PRO}:ARF5:GFP or BES1\textsubscript{PRO}:BES1:GFP containing chromatin fragments. Samples were washed and eluted off of Dynabeads using nuclei lysis buffer, and cross-links were reversed by incubating with 300 mM NaCl. DNA was purified using a PCR clean-up kit (28104; Qiagen). Low adhesive DNase/RNase free tubes (B74030; Bioplastics) were used for all the procedures. ChiP-qPCR assay data were normalized to housekeeping gene (At1g13320) coding sequence, and results are expressed as ratios of qPCR signal to the antibody IP of wild-type samples (Figure 1) or IP without antibody in reporter lines (Figure S1). ChiP-qPCR results represent the average of at least 2–4 independent biological replicates. Primers for ChiP-qPCR analysis are listed in Table S2.

3 | RESULTS

3.1 | BES1 sensitizes hypocotyl to exogenous application of auxin

Brassinosteroids (BRs) enhance seedling sensitivity to auxin (Vert et al., 2008). To test the extent to which this effect of BR on auxin response is mediated by BES1, we exposed seedlings with wild type or constitutively active BES1 to the synthetic auxin picloram after 3 hr, similar to sample preparation for RT-qPCR. GUS staining was performed as previously described (Lilley, Gee, Sairanen, Ljung, & Nemhauser, 2012) using 1 mM Ferri/Ferro concentration for 1.5 hr at 37°C in the dark. Seedlings were mounted on glass slides in 50% glycerol. Images were taken using Leica microscope, and whole seedling images were reconstructed using MosaicJ feature of Fiji plugin from National Institutes of Health ImageJ software (rsb.info.nih.gov).
BES1 increases auxin sensitivity and DNA binding by ARF5. (a) Hypocotyl response of wild-type (WT) and bes1-D gain-of-function mutant seedlings to the synthetic auxin picloram (PIC). Open symbols are seedlings exposed to control treatments, and closed symbols are hormone-treated samples. bes1-D was significantly taller than WT on D4 under mock and treated conditions. bes1-D was significantly longer than WT on D4-8. Asterisks indicate p value < .001 (by Student’s t test comparing mutant to wild type in the same treatment). (b) Binding by ARF5 on several promoters was enhanced in bes1-D mutants under mock condition. Bars represent the mean of four biological replicates (Figure 1a). As previously described, bes1-D hypocotyls were longer than those of wild-type seedlings (Figure 1a; Yin et al., 2005); however, the application of synthetic auxin, picloram, strongly exaggerated the difference in hypocotyl length between bes1-D mutant and wild-type plants. Based on previous results using the SAUR15 promoter (Walcher & Nemhauser, 2012), we hypothesized that increased BES1 activity led to auxin hypersensitivity by enhancing DNA binding of ARF5 to promoters with bipartite-type cis-elements. Using the large list of BES1 targets generated by ChIP-seq and microarray analysis (Yu et al., 2011), we focused on the subset of targets with putative bipartite elements in their promoters. Ten genes were selected based on the following criteria: being targets of ARF5 or BES1 (Schlereth et al., 2010; Yu et al., 2011), having evidence of their expression regulated by auxin or BRs (Goda et al., 2004; Nemhauser et al., 2004), and functional information and/or mutant phenotypes. We validated these candidate genes and the bipartite elements in their promoters with ChIP assays in the previously characterized ARF5::ARF5:GFP (Schlereth et al., 2010) and BES1::BES1::GFP (Yin et al., 2002) lines. From our initial list, Xyloglucan Endotransglucosylase/Hydrolase 19 (XTH19), Indole-3-Acetic Acid 6 (IAA6), Longifolium 1 (LNG1), and Phytochrome-Interacting Factor 7 (PIF7) were found to be the strongest candidates for further analysis (Figure S1). To test whether BES1 enhances DNA binding of ARF5, we crossed ARF5::ARF5:GFP line to bes1-D mutant. Increased BES1 activity did increase DNA binding by ARF5 in most cases (Figure 1b).

3.2 | BES1 and BEH4 are major regulators of BR responses in seedling stage

Brassinosteroid-Insensitive1-EMS-Suppressor1/Brassinozoleresistant 2 belongs to a family of six genes (Yin et al., 2005), with BES1 and BZR1 the best-characterized members (Sun et al., 2010; Yu et al., 2011). BEH1-4 undergo BR-induced phosphorylation status changes similar to BES1 and BZR1 (Yin et al., 2005). The functional redundancy of this transcription family was documented recently in trait robustness (Lachowiec et al., 2018); however, their role in BR pathways remains poorly understood. BEH4, the most recent member of the family, acts redundantly with BES1 to regulate hypocotyl length in skotomorphogenic seedlings (Lachowiec et al., 2018). To investigate whether other members of the BES1/BZR1/BEH gene family contribute to auxin sensitivity, we analyzed T-DNA insertion alleles for each member of the BES1/BZR1/BEH family (Lachowiec et al., 2018). In these T-DNA insertion lines, we measured hypocotyl elongation in the absence or presence of BL or picloram. Hypocotyl response to BL was modestly reduced only in bes1-2, bsr1-2, and beh4-1 single mutants (Figure S2), while the response to picloram was not affected significantly in any of single mutants (Figure S3). Based on these findings, we selected bes1-2 beh4-1 and bes1-2 bsr1-2 double mutants for further investigation. bes1-2 beh4-1 double mutants are dwarfs that resemble known mutants with compromised BR synthesis or signaling (Figure 2a). They also show dramatically reduced response to BL and significantly reduced response to picloram (Figure 2b,c). In addition, rosettes of bes1-2 beh4-1 resembled bin2-D mutants where activity of the entire BES1/BZR1/BEH family should be suppressed (Figure 2a). In contrast, bes1-2 bsr1-2 double mutants had an essentially wild-type response to picloram, but significant reduction in BR sensitivity (Figure S4).

3.3 | BES1 status affects bipartite gene expression

We used RT-qPCR to investigate the effect of BES1 on bipartite target gene expression (XTH19, IAA6, LNG1, and PIF7). Single mutants of bes1-2 and beh4-1 did not have significant effect on target gene expression (Figure S5), consistent with their weak physiological phenotypes (Figures S2 and S3). However, in bes1-2 beh4-1 double mutant the expression of XTH19 and IAA6 was attenuated, and
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As expected, in bes1-D and beh4-1, PIF7 with the wild type. In contrast, the expression of LNG1 was modestly decreased in comparison to control treatments, and closed symbols are hormone-treated samples. bes1-2 beh4-1 control samples are the same and repeated for reference in panels b and c. Significance was assessed using Student’s t test comparing mutant to wild type in the same treatment.

For BEH4 acts redundantly with BES1 to regulate auxin and BR seedling responses. (a) Seven-day-old seedling (upper panel) and 6-week-old rosette (lower panel) phenotype of wild-type, bin2-D, bes1-2 beh4, bes1-2, and beh4-1 mutants grown in short-day conditions. Hypocotyl response of wild-type (WT) and bes1-2 beh4-1 mutant seedlings to (b) brassinolide (BL) and (c) picloram (PIC), grown as indicated in Figure 1. Open symbols are seedlings exposed to control treatments, and closed symbols are hormone-treated samples. bes1-2 beh4-1 was significantly shorter than WT on D4-8 (p value < .01) under control and brassinolide, and only on D7-8 (p value < .05) picloram conditions. Asterisks indicate p values. WT and bes1 beh4-1 control samples are the same and repeated for reference in panels b and c. Significance was assessed using Student’s t test comparing mutant to wild type in the same treatment.

3.4 | Evolutionary conservation of bipartite elements predicts hormone responsiveness of promoters

XTH19, IAA6, and LNG1, as well as the previously characterized bipartite target gene SAUR15, belong to large gene families. In the case of XTH19, we observed that the expression of at least three genes in the same clade (XTH17-19) was similarly affected by BES1, as well as treatment with BR or auxin (Figure 4b,c). We reasoned that these similar transcriptional patterns across paralogs might be due to shared promoter architecture. Previously, it was documented that XTH17-20 cluster has a specific motif conservation within the promoter region (Vissenberg et al., 2005). We found that this conserved motif includes the bipartite element we found in XTH19 promoter (Figure 4a). Similar promoter architecture is also observed for group of so-called SAUR classes 2 and 3 that are expressed in hypocotyls (Sun et al., 2016). 17 out of 30 genes in this group harbor bipartite elements within 250bp upstream of the transcription start, and all of these have been shown to be regulated by BR and auxin (Table S1). We predicted that the absence of bipartite element would compromise hormone responsiveness of target genes. In fact, no hormonal response to either BL or IAA was observed for several XTHs closely related to XTH17-19 cluster, such as XTH14, XTH31, and XTH32, in which either HUD or AuxRE elements were lost. XTH31 expression was still responsive to BL, likely due to a retained HUD element at −900 bp. XTH26 has conserved bipartite element and was included as a positive control. Despite its low expression levels, XTH26 was repressed by both hormones (Figure 5).

In previous work focused on characterization of the XTH17-20 cluster, Vissenberg and colleagues generated a set of transgenic lines of promoter truncations fused to GUS. We tested full-length (XTH19PRO-1.1kb::GUS) and 300bp (XTH19PRO-0.3kb::GUS) truncated promoter reporter lines in our conditions (Figure 5). Seedlings of full-length XTH19PRO-1.1kb::GUS line exhibited weak staining in the hypocotyl area and root tip in control (mock) conditions, 3 hr of IAA

the opposite tendencies were observed for all genes (Figure 3a). Both BR and auxin dramatically induced the expression of XTH19 and IAA6, while both hormones modestly repressed the expression of PIF7 (Figure 3b). The hormone responsiveness of those genes was affected by BES1/BEH4 status (Figure 3c).

We further investigated the role of bipartite target genes in BR and auxin-induced elongation responses. We analyzed single knockout alleles of xth17, lng1-3, and pif7-2. Since XTH19 had no available loss-of-function mutant, we analyzed a mutant in XTH17, part of the same XTH17-20 gene cluster whose members act redundantly with one another (Miedes et al., 2013; Vissenberg et al., 2005). XTH17 promoter also carried a conserved bipartite element, and its expression followed a similar pattern as XTH19 in our RT-qPCR analysis (Figure 4b). The hypocotyls of all three mutants tended to be shorter than those in wild-type seedlings in control (mock) conditions. In the presence of picloram, these effects became more obvious (Figure 3d).
and BL treatment intensified the blue staining specifically in hypocotyl and petiole tissues. Interestingly, the XTH19\textsubscript{PRO-0.3kb}::GUS truncation, in which a portion of the bipartite elements is deleted, completely abolished reporter gene expression and hormone responsiveness (Figure S6). These data suggest that bipartite elements in \textit{XTH19} promoter are important for its proper expression level, pattern, and hormone sensitivity.

4 | DISCUSSION

During photomorphogenesis, seedling growth is shaped by a complex interacting networks of plant hormones to insure the seedling architecture and growth are synchronized to environmental conditions. In this study, we provide molecular mechanism for how two well-characterized regulators of photomorphogenesis, auxin and BRs, converge in a tunable bipartite transcriptional module to promote growth.

We have shown that \textit{BES1} and \textit{BEH4}, but not \textit{BZR1}, play a major role in BR and auxin responsiveness in young seedlings. These results are consistent with a recent study that found that \textit{BEH4} acts redundantly with \textit{BES1} in controlling hypocotyl length robustness in dark-grown seedlings (Lachowiec et al., 2018). \textit{BES1} is also temperature sensitive, a trait regulated by auxin (Heggie & Halliday, 2005; Lachowiec et al., 2013). It is likely that other family members, such as \textit{BZR1}, play growth-promoting roles at other stages or under other conditions. Consistent with this hypothesis, ChIP-seq analyses of \textit{BES1} and \textit{BZR1}, performed in two-week-old seedlings and leaves of adult plants, respectively, found a large overlap in target promoters (Sun et al., 2010; Yu et al., 2011). \textit{BES1} and \textit{BZR1} show differential interaction with the chaperone HSP90 (Lachowiec et al., 2013) which may facilitate interactions with distinct cell type- or stage-specific partners.

Cooperative binding and activity of BR-regulated transcription factors appear to be a recurrent motif in plant signaling. Our group previously found that the interaction between auxin and BR relies on two cis-elements, a bipartite element that contains AuxRE and HUD-type E-box elements bound by ARF5 and \textit{BES1}, respectively (Walcher & Nemhauser, 2012). Here, we show that specific mutations of E-box/AuxRE elements during evolution or disruption of bipartite target \textit{XTH19\textsubscript{PRO}} had direct effect on gene expression level and patterns (Figure 5 and Figure S6). A similar mechanism of
Cooperative binding has been proposed for BZR1 and ARF6 (Oh et al., 2014). BES1 also interacts with the bZIP transcription factors HAT1 and HAT3 to co-repress the BR biosynthetic gene DWF4 (Zhang et al., 2014). In addition, both BES1 and BZR1 interact with the bHLH transcription factor PIF4 and bind to E-box elements to co-activate target promoters (Oh, Zhu, & Wang, 2012).
genes to specific environmental conditions

module to connect expression of a suite of growth-promoting ARF5, BES1, and BEH4 to work together as a transcriptional molecular switch to integrate signals. Bipartite elements allow ARF5–BES1–BEH4 transcriptional hub acts as a FIGURE 6

GROWTH PROGRAM

Bipartite elements allow ARF5, BES1, and BEH4 to work together as a transcriptional module to connect expression of a suite of growth-promoting genes to specific environmental conditions.

Target genes with bipartite promoters identified in this study are known regulators of plant growth. IAA6, also called Short Hypocotyl 1, encodes a co-repressor of auxin signaling that is also involved in negative feedback (Overvoorde, 2005). PIF7 is a major regulator of shade responses that directly induces auxin biosynthesis genes such as members of the YUCCA family (Li et al., 2012). Dominant iaa6/shy1-1D and loss-of-function pif7 mutants exhibit short hypocotyl phenotypes (Kim, Soh, Kang, Furuya, & Nam, 1996; Li et al., 2012) (Figure 3d). LNG1 encodes a protein localized to cortical microtubules (cMT) (Drevensek et al., 2012) that was initially identified as a dominant mutant with exaggerated elongation of petioles via unidirectional cell elongation (Lee et al., 2006; Sapala et al., 2018).

The switch between different cMT orientations can be triggered by various endogenous and exogenous signals that are known to modulate growth, including light and various hormones (Chen, Wu, Liu, & Friml, 2016; Robinson & Kuhlemeier, 2018; Wu & Bezanilla, 2018). Actin and cMT proper orientation and localization are required for both auxin and BR-mediated cell elongation (Lanza et al., 2012; Sasidharan et al., 2014; Wang et al., 2012). In addition, Sasidharan and colleagues demonstrated that genetic and pharmacological disruption of cMTs affects a shade-specific subset of XTH gene (XTH17 and XTH19) expression as a result of auxin re-distribution (Sasidharan et al., 2014). XTHs, similar to expansins, are cell wall modifiers that induce cell expansion. Overexpression of XTH18, XTH19, and XTH20 (all part of the bipartite XTH clade) stimulated hypocotyl growth in early developmental stage of Arabidopsis seedlings (Miedes et al., 2013), while similarly loss of function of XTH17 results in inhibition of hypocotyl growth (Figure 3d). In addition, the functionally redundant LNG3 and LNG4 genes regulate turgor-driven polar cell elongation through activation of XTH17 and XTH24 (Lee et al., 2018). BES1/BZR1 and PIFs were also implicated in hypocotyl elongation during thermomorphogenesis via regulation of LNG1 and LNG2 (Hwang et al., 2017; Ibañez et al., 2018; Li et al., 2018). We found that LNG1 and XTH19 are bipartite targets of the ARF5–BES1/BEH4 module rapidly induced by both auxin and BRs (Figures 3 and 4) further connecting reorientation of cMTs and cell wall loosening.

We propose that the ARF5–BES1/BEH4 transcriptional hub rapidly and coordinately modulates a suite of growth control genes (Figure 6). This module can serve as an integration point for external signals such as shade and temperature to tune internal growth program and adjust it to a changing environment. For example, exposure to shade could rewire this growth network by increasing the expression/activity of bHLH transcription factors that interact with BES1, and, in this way, change the composition and thereby the targets of the auxin/BR transcriptional complex. Future studies are needed to fully understand how each potential transcriptional complex impacts target gene selectivity and growth dynamics in a tissue- and developmental stage-specific manner.
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CONFLICT OF INTEREST

The authors declare no conflict of interest associated with the work described in this manuscript.

AUTHORS CONTRIBUTION

AG and JLN conceived and designed the experiments. AG collected and analyzed the data, as well as preparing the figures. AG and JLN wrote the manuscript.

REFERENCES


SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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