

Bipartite Promoter Element Required for Auxin Response^{1[C][W][OA]}

Cristina L. Walcher and Jennifer L. Nemhauser*

Department of Biology, University of Washington, Seattle, Washington 98195–1800

Multiple mechanisms have been described for coordination of responses to the plant hormones auxin and brassinosteroids (Zhang et al., 2009). One unexplained phenomenon is the reliance of the auxin transcriptional response on a functional brassinosteroid pathway. In this study, we used luciferase reporters to interrogate the promoter of *SMALL AUXIN-UP RNA15* (*SAUR15*), a well-characterized auxin and brassinosteroid early response gene in *Arabidopsis* (*Arabidopsis thaliana*). After identifying a minimal region sufficient for auxin response, we targeted predicted cis-regulatory elements contained within this sequence and found a critical subset required for hormone response. Specifically, reporter sensitivity to auxin treatment required two elements: a Hormone Up at Dawn (HUD)-type E-box and an AuxRE-related TGTCT element. Reporter response to brassinosteroid treatment relied on the same two elements. Consistent with these findings, the transcription factors BRASSINOSTEROID INSENSITIVE1-EMS SUPPRESSOR1 and MONOPTEROS (MP)/AUXIN RESPONSE FACTOR5 (ARF5) showed enhanced binding to the critical promoter region containing these elements. Treatment with auxin or brassinosteroids could enhance binding of either transcription factor, and brassinosteroid enhancement of MP/ARF5 binding required an intact HUD element. Conservation of clustered HUD elements and AuxRE-related sequences in promoters of putative *SAUR15* orthologs in a number of flowering plant species, in combination with evidence for statistically significant clustering of these elements across all *Arabidopsis* promoters, provided further evidence of the functional importance of coordinated transcription factor binding.

Auxin and brassinosteroids are essential for photomorphogenesis in *Arabidopsis* (*Arabidopsis thaliana*) and promote growth in many plant species (Hardtke et al., 2007; Stewart and Nemhauser, 2010). Microarray studies have identified a large number of early response genes shared by both hormones (Goda et al., 2004; Nemhauser et al., 2006). Many of these common targets are synergistically induced when both hormones are provided exogenously (Nemhauser et al., 2004; Vert et al., 2008). In addition, auxin and brassinosteroid transcriptional response pathways also exhibit interdependence; changes in gene expression in response to one hormone require the function of the other (Nemhauser et al., 2004, 2006; Nakamura et al., 2006; Hardtke, 2007).

The close relationship between auxin and brassinosteroids likely reflects several levels of cross-regulation.

For example, brassinosteroids have been shown to promote polar auxin transport, leading to altered auxin distribution within and between organs (Bao et al., 2004; Li et al., 2005). In addition, auxin regulation of brassinosteroid biosynthesis is required to sustain a robust auxin response in diverse tissues (Mouchel et al., 2006; Beuchat et al., 2010). Bioinformatic analyses of target genes for each hormone suggest that auxin and brassinosteroid could directly affect one another's signaling pathways (Goda et al., 2004; Nemhauser et al., 2006). Recent studies have supported this model with evidence for cross-regulation of both auxin- and brassinosteroid-associated transcription factors (Nakamura et al., 2003b, 2006; Kim et al., 2006; Vert et al., 2008; Chandler et al., 2009). How these interactions manifest in transcriptional complexes on target promoters remains largely unknown.

The transcription factors regulated by auxin and brassinosteroids are well characterized, as are their cognate cis-regulatory elements. The AUXIN RESPONSE FACTOR (ARF) family of transcription factors binds to auxin-responsive elements with the sequence TGTCTC, called canonical AuxREs (Ulmasov et al., 1995) or ARFATs (Higo et al., 1999). A repression complex that can be removed in the presence of auxin modulates ARF activity (Gray et al., 1999, 2001; Tiwari et al., 2001; Szemenyei et al., 2008). Brassinosteroids change gene expression by activating the BRASSINAZOLE RESISTANT1 (BZR1; Wang et al., 2002)/BRASSINOSTEROID INSENSITIVE1 (BRI1)-EMS SUPPRESSOR1 (BES1; Yin et al., 2002) family of transcription factors. BES1 and BZR1 have been shown to bind to E-boxes

¹ This work was supported by the National Science Foundation (grant no. IOS-0919021) and the University of Washington. C.L.W. was supported by the Developmental Biology Predoctoral Training Grant (T32HD007183) from the National Institute of Child Health and Human Development.

* Corresponding author; e-mail jn7@uw.edu

The author responsible for distribution of materials integral to the findings presented in this article in accordance with the policy described in the Instructions for Authors (www.plantphysiology.org) is: Jennifer L. Nemhauser (jn7@uw.edu).

^[C] Some figures in this article are displayed in color online but in black and white in the print edition.

^[W] The online version of this article contains Web-only data.

^[OA] Open Access articles can be viewed online without a subscription.

www.plantphysiol.org/cgi/doi/10.1104/pp.111.187559

(CANNTG; Yin et al., 2005; Sun et al., 2010; Yu et al., 2011) and to a Brassinosteroid Response Element [BRRE; CGTG(T/C)G; He et al., 2005; Sun et al., 2010; Yu et al., 2011). Brassinosteroids activate BZR1/BES1 family members by binding to the receptor-like kinase BRI1 and triggering a signaling cascade that ultimately inhibits phosphorylation of BES1 and BZR1 (Kim et al., 2009; Kim and Wang, 2010).

Promoters of auxin-brassinosteroid shared target genes contain putative binding sites for transcription factors relevant to each hormone pathway (Goda et al., 2002; Müssig et al., 2002; Yin et al., 2002; Nemhauser et al., 2004), and some of these sites have been confirmed in vivo (Yin et al., 2005; Cole et al., 2009). However, not all auxin-responsive genes contain the canonical TGTCTC AuxRE (Nemhauser et al., 2004), leading to the suggestion that AuxRE variants may also be functional (Guilfoyle and Hagen, 2007; Chapman and Estelle, 2009). To date, no direct interactions between ARFs and BES1/BZR1 have been reported, and there is little information about how known elements relate to hormone interdependence.

In this study, we used the promoter of *SMALL AUXIN-UP RNA15* (*SAUR15*) to dissect the mechanism for auxin reliance on brassinosteroids for induction of target genes. *SAUR15* belongs to one of the first identified families of auxin early response genes (McClure et al., 1989) and has been studied extensively as a brassinosteroid early response gene (Yin et al., 2005; Vert et al., 2008; Sun et al., 2010; Yu et al., 2011). Here, we show that auxin and brassinosteroid responses require the same cis-regulatory elements: a Hormone Up at Dawn (HUD)-type E-box in combination with a nearby AuxRE variant. BES1 and MONOPTEROS (MP)/ARF5 bind to the promoter region containing these sites, and binding of either transcrip-

tion factor can be enhanced by treatment with either auxin or brassinosteroids. Analysis of putative orthologs from diverse species reveals conservation of clustered AuxRE variants and HUD elements, and a number of other key Arabidopsis genes in the auxin and brassinosteroid pathways share similar promoter architecture. This work points to an additional mechanism of coordination between auxin and brassinosteroid transcriptional responses.

RESULTS

SAUR15 Is Induced by Auxin and Brassinosteroid Treatment

Growth promotion by auxin or brassinosteroids requires the function of both signaling pathways (Nakamura et al., 2003a, 2003b, 2006; Nemhauser et al., 2004). It is less clear if this phenomenon reflects a convergence at the level of transcriptional control (Wang et al., 2005). To determine whether auxin and brassinosteroids coordinately regulate activity on target gene promoters, we analyzed the regulatory region of *SAUR15*, one of the best characterized auxin- and brassinosteroid-responsive genes (Gil et al., 1994; Gil and Green, 1996, 1997; Nakamura et al., 2003a). *SAUR15* is rapidly induced following hormone treatments (Gil et al., 1994; Nakamura et al., 2003a). In our conditions, *SAUR15* mRNA levels more than quadrupled within 1 h of auxin or brassinosteroid treatment (Fig. 1A). As previously reported, response dynamics differed somewhat between the two hormones (Nakamura et al., 2003a). Seedlings treated with the natural auxin indole-3-acetic acid (IAA) sustained a similar level of mRNA induction after 1 or 3 h. For seedlings treated

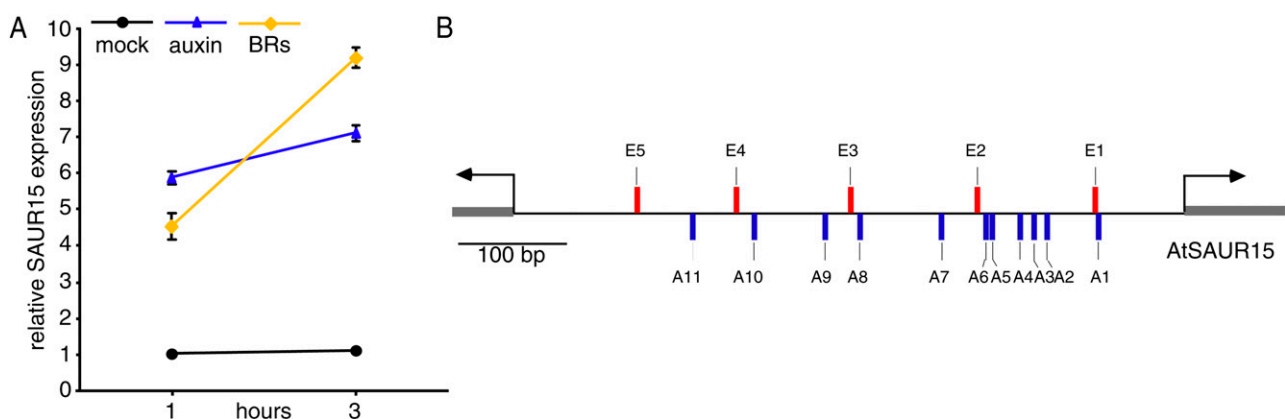


Figure 1. *SAUR15* is an auxin- and brassinosteroid (BR)-responsive gene. A, *SAUR15* is responsive to both auxin and brassinosteroids. Quantitative RT-PCR analysis was performed on three independent biological replicates of total RNA isolated from seedlings following treatment with mock, auxin, or brassinosteroids for 1 or 3 h. Expression at 1 h under mock treatment is set to 1. Error bars represent SE. B, The intergenic region between *At4g38840* and *At4g38850* (*SAUR15*) contains a number of cis-regulatory elements important for response to auxin and brassinosteroids. Red bars located above the line represent predicted or known brassinosteroid cis-regulatory elements: E1-E5, E-boxes. Blue bars located below the line represent potential auxin cis-regulatory elements: A1, AuxRE variant (TGTGCTC); A2-A11, TGTC/GACA cores; A5, canonical AuxRE (TGTCTC). [See online article for color version of this figure.]

with the brassinosteroid brassinolide, mRNA levels were clearly elevated within 1 h and were consistently higher after 3 h (Fig. 1A).

SAUR15 (*At4g38850*) is an obvious target for analysis of regulatory regions. Similar to other *SAUR* genes, it contains no introns, and there is only a small upstream intergenic region shared by *At4g38840*. Within the putative *SAUR15* promoter sequence, there are several predicted auxin and brassinosteroid cis-regulatory elements (Fig. 1B). For brassinosteroids, there are five E-boxes (Fig. 1B, red ticks numbered E1–E5) but no BRRE elements. E1, E2, E3, and E5 are HUD elements with the sequence CACATG/CATGTG (Michael et al., 2008). HUD elements were previously reported as overrepresented in the promoters of cycling genes related to diverse hormone pathways (Michael et al., 2008). Previous studies have shown that MYB30 can directly interact with BES1, bind to a site immediately adjacent to E4, and increase induction of *SAUR15* expression (Li et al., 2009). For auxin, there are 10 TGTC/GACA core elements (Fig. 1B, blue ticks numbered A2–A11), including one canonical AuxRE (GA-GACA; A5). A1 overlaps with E1 and represents an AuxRE variant with a single base insertion in the middle of the element (TGTGCTC). A related element found in the promoter of the brassinosteroid biosynthetic gene *DWF4* was recently shown to be important for auxin response (Chung et al., 2011).

A Short Promoter Region Is Sufficient for Normal Auxin Response and Localization of *SAUR15*

To identify a short promoter sufficient for auxin response, we assayed a series of deletions of the intergenic region upstream of *SAUR15*. These putative promoter regions were used to drive expression of a

luciferase reporter (LUC). First generation transformed (T1) seedlings were presprayed with luciferin to remove accumulated LUC enzyme and subsequently exposed to auxin for 3 h. Auxin response for each construct was determined by averaging fold-change for a large number of T1 seedlings (24 independent transformants for *pS15-5E* and 30 independent transformants for all other constructs). When the reporter was driven by the full intergenic region upstream of *SAUR15* (*pS15-5E*), seedlings showed similar auxin response to previous studies with the well-characterized *SAUR-AC1* reporter (Gil and Green, 1997; Fig. 2A). A reduction of the putative promoter to 290 bp [*pS15-2E(ii)::LUC*] resulted in a modest increase in auxin sensitivity, suggesting there may be negative regulators that can bind these upstream regions. The slightly longer *pS15-2E(i)::LUC* reporters showed similar auxin response to the full-length constructs. The additional sequence included in this reporter construct does not contain any TGTC/GACA cores, so any negative regulation is unlikely to be ARF mediated. Auxin response of reporter constructs with only 200 bp of the upstream regulatory region resembled full-length constructs, suggesting they retained core sites required for hormone sensitivity [Fig. 2A; *pS15-1E(i)::LUC*]. A further reduction of the promoter by 100 bp [*pS15-1E(ii)::LUC* and *pS15-0E::LUC*] resulted in a complete loss of auxin response.

To test whether our deletions changed the pattern of *SAUR15* expression, we engineered additional seedlings with the *pS15-5E*, *pS15-2E(ii)*, and *pS15-1E(i)* regulatory regions driving expression of the *uidA* gene encoding GUS. The original *SAUR-AC1::GUS* reporter included the intergenic region between *SAUR15* and *At4g38840*, the entire coding region of *At4g38840*, and part of the coding region of *At4g38830*. All of the shorter

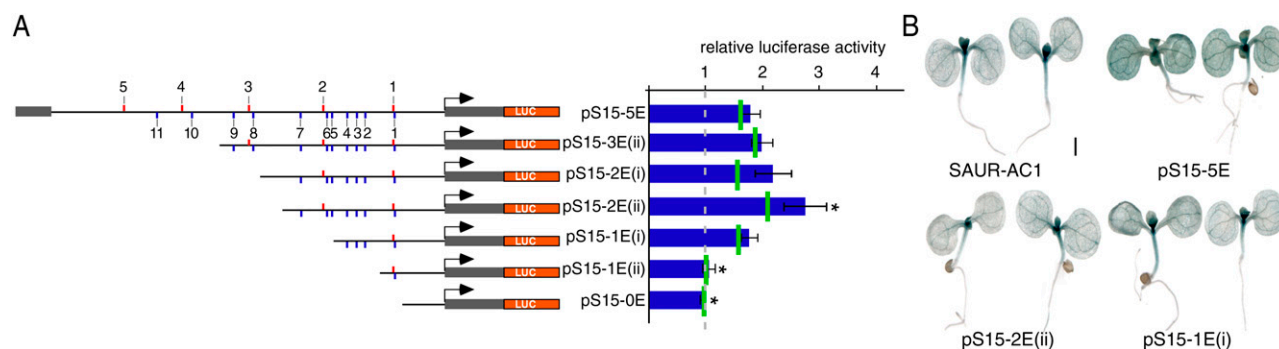


Figure 2. A short region is required for auxin sensitivity and localization of *SAUR15*. A, Auxin response requires a region containing three TGTC/GACA cores (A2–4), but not the A5 element. Relative luciferase activity is defined as activity after 3 h divided by baseline. Median response levels are indicated by horizontal green lines. Linear regression was used to test for significant differences in auxin response between constructs. Asterisks indicate lines with significant differences in auxin response from *pS15-5E*. *pS15-2E(ii)* showed significantly higher auxin response, while *pS15-1E(ii)* and *pS15-0E* showed a significantly lower auxin response. Twenty-four independent transformants were evaluated for *pS15-5E*. At least 30 T1 seedlings were analyzed for all other lines. Error bars represent SE. B, Expression pattern of *SAUR15* is retained in short auxin-responsive reporter lines. Deletion constructs with auxin response [*pS15-5E::GUS*, *pS15-2E(ii)::GUS*, or *pS15-1E(i)::GUS*] show similar expression pattern as the original *SAUR-AC1::GUS* reporter. For all seedlings, staining is highest in the petioles of young leaves and the hypocotyl region closest to the shoot apical meristem. Staining can also be detected in the vasculature of cotyledons. No staining is detected in the roots. Scale is the same in all panels; bar represents 1 mm.

constructs we analyzed showed similar staining patterns to seedlings containing the original *SAUR-AC1* construct (Fig. 2B). While there may be functionally important sequences outside of the minimal region defined here, the 200-bp region in the *pS15-1E(i)* construct was sufficient for both auxin response and proper localization of reporter expression.

Auxin and Brassinosteroid Induction of *SAUR15* Requires Two Crucial Elements

To directly assess the contribution of specific cis-regulatory elements to auxin response, we systematically mutated TGTC/GACA cores (A1–A5) and E-boxes (E1–E2) in the *pS15-2E(ii)::LUC* construct. The *pS15-2E(ii)::LUC* construct was selected for further analysis because it showed the lowest levels of LUC activity in the absence of hormones (Supplemental Fig. S1) and a robust increase in auxin-induced promoter activity (Fig. 2A). The low basal level of reporter expression reduced noise in the luciferase assays and allowed detection of smaller effects on reporter activity. We began our analysis with the canonical AuxRE (TGTC, A5) and its close variant (TGTGCTC, A1) because these seemed the most likely to confer auxin responsiveness (Chung et al., 2011). Contrary to this expectation, neither A5 nor A1 were essential for auxin response, although A5 did contribute to the magnitude of the induction by auxin (Fig. 3A). In contrast, most T1 seedlings transformed with reporters lacking the A2, A3, and A4 TGTC/GACA cores [*pS15-2E(ΔA4A3A2)::LUC*] showed a complete loss of auxin response. Loss of A2 alone [*pS15-2E(ΔA2)::LUC*] drastically reduced auxin induction of LUC activity, demonstrating that A2 is acting as an essential AuxRE in the *SAUR15* promoter. Recently, another element with the same sequence as A2 (CAGACA) was found to act as a bona fide AuxRE in the unrelated *ATHB8* promoter (Donner et al., 2009). Consistent with this finding, we observed that loss of A2 alone [*pS15-2E(ΔA2)::LUC*] drastically reduced auxin induction of the reporter, demonstrating that A2 (CAGACA) but not A5 (GAGACA) is the AuxRE for *SAUR15*.

We also tested whether E-boxes, which have been linked to brassinosteroid responses, had any role in auxin effect on transcription. Surprisingly, mutations in both E-boxes closest to the transcriptional start site [*pS15-2E(ΔE2E1)::LUC*] eliminated auxin response. Loss of E1 alone [*pS15-2E(ΔE1)::LUC*] but not E2 [*pS15-2E(ΔE2)::LUC*] was sufficient for this effect. Previous work on the soybean (*Glycine max*) *SAUR15A* gene (Xu et al., 1997) identified an auxin-responsive domain containing an E-box but no AuxRE-related sequence. Our results, in combination with this previous work, lend functional significance to computational studies showing a significant overrepresentation of E-boxes in shared auxin and brassinosteroid targets (Nemhauser et al., 2004). It is also worth noting that constructs with a reduced response to auxin treatment

showed somewhat lower basal levels of luciferase activity (Supplemental Fig. S1), perhaps reflecting a reduced response to endogenous hormone levels. Another possibility is that fluctuations in basal levels among reporter lines could reflect the impact of additional regulatory pathways acting on *SAUR15* or additional modulators of auxin response.

Because previous studies had demonstrated auxin dependence on brassinosteroids, we wanted to check whether this relationship extended to specific cis-elements. Two T1 plants representing median auxin responses were selected for each reporter construct. By using isogenic lines rather than individual T1 plants, we could expose presprayed seedlings to mock or hormone treatments and measure luciferase activity at the identical time point. In this way, we could eliminate any potential effect of the circadian clock or extended duration of submergence, while also directly comparing reporter responses for each hormone. Auxin response in all T2 seedlings was similar to what was observed for the same constructs in the T1 experiments, suggesting that neither the circadian clock nor extended duration of submergence interfered with measurements of auxin response (Fig. 3B).

Seedlings from T2 families carrying *pS15-2E(ii)::LUC*, as well as those containing mutations in predicted cis elements, were exposed to 3 h of mock, auxin, or brassinosteroid treatments (Fig. 3B). Unlike in the T1 analysis, average relative luciferase activity for each T2 family was determined by dividing the average luciferase activity of all hormone-treated seedlings by the average luciferase activity of all mock-treated seedlings. Similar to the results in the T1 plants, mutations in A2 or E1 substantially reduced auxin response (Fig. 3B). Brassinosteroids had a more subtle effect on reporter induction than auxin, reducing the power to detect statistically significant hormone effects. Despite this limitation, brassinosteroid response did follow the same pattern as auxin response, requiring both A2 and E1 sites (Fig. 3B).

Treatment with Either Auxin or Brassinosteroids Can Enhance DNA Binding by Key Transcription Factors

E-boxes and AuxRE elements have been linked with brassinosteroid and auxin responses, respectively. Chromatin immunoprecipitation (ChIP) assays have shown that BES1 can bind the promoter of *SAUR15* in the region around E5 and E4 (Yin et al., 2005). This area is approximately 500 bp upstream of the transcriptional start site and outside of the minimum region defined here. We performed additional ChIP assays on *pBES1::BES1:GFP* seedlings to test for binding near the A2 and E1 sites. Brassinosteroid treatment caused a substantial enrichment in BES1 binding in this region (Fig. 3C). This finding is consistent with recent global ChIP data showing BES1 binding enriched near transcriptional start sites (Yu et al., 2011). BES1 binding further upstream, perhaps in conjunction with MYB30, is not essential but may enhance brassinosteroid re-

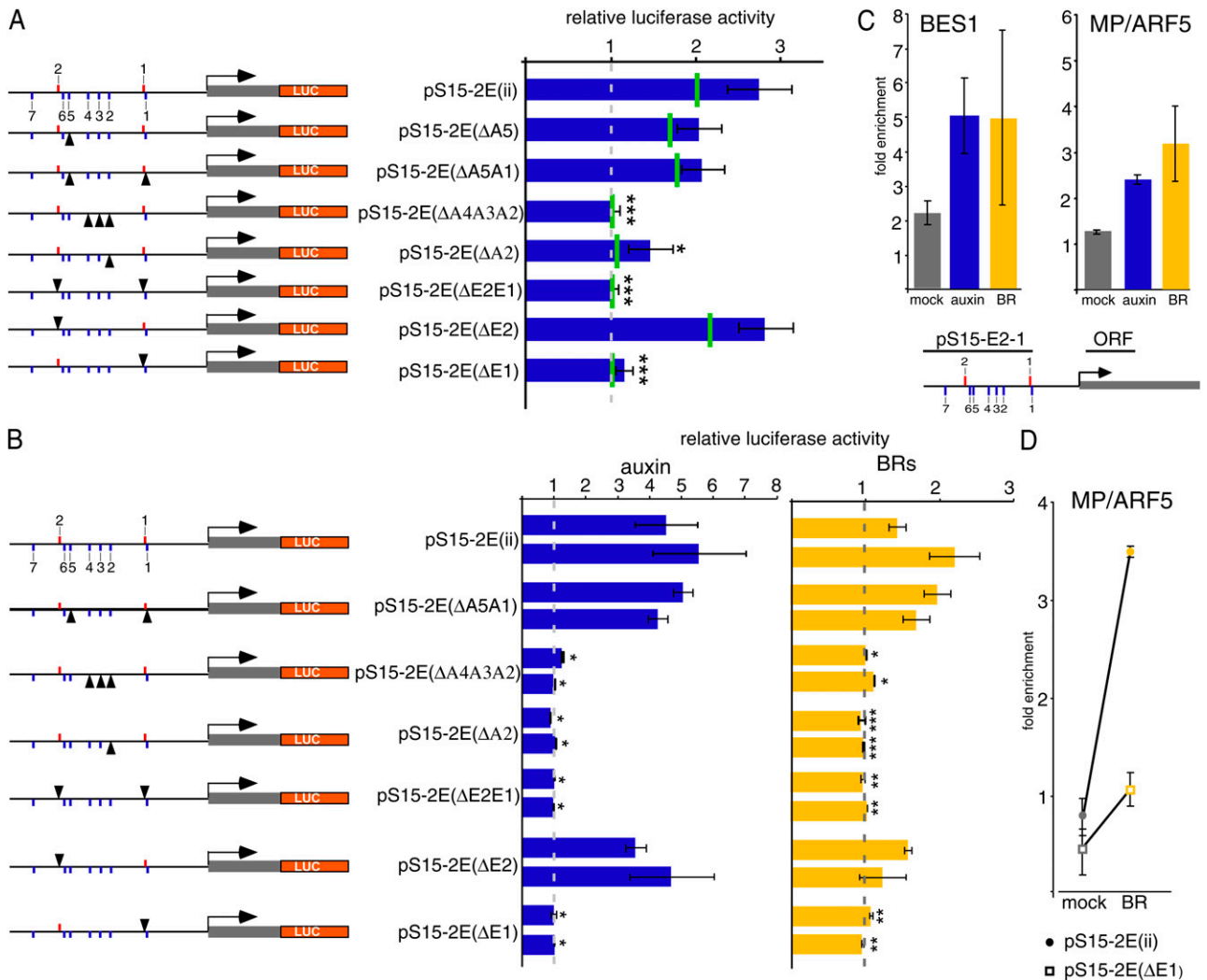


Figure 3. cis-Regulatory elements are required for auxin and brassinosteroid response. **A**, A2 and an E1 are required for auxin response. Relative luciferase activity is defined as activity after 3 h divided by baseline. Median response levels are indicated by horizontal green lines. Linear regression was used to test for significant differences in auxin response between constructs. Asterisks indicate lines with significant differences in auxin response from *pS15-2E(ii)*. Loss of the canonical AuxRE [*pS15-2E(ΔA5)*] led to a modest albeit statistically significant reduction in auxin response. Loss of A2, A3, and A4 [*pS15-2E(ΔA4A3A2)*] or A2 alone [*pS15-2E(ΔA2)*] essentially eliminated auxin response. Loss of E1 alone [*pS15-2E(ΔE1)*] or in combination with a loss in E2 [*pS15-2E(ΔE2E1)*] led to a similarly severe loss of reporter sensitivity to auxin treatment. At least 30 T1 seedlings were analyzed for all lines. Error bars represent SE. **B**, Brassinosteroid response follows a similar pattern as auxin response. Two representative T1 individuals carrying each construct were selected for propagation. Luciferase activity of 24 seedlings from each T2 line was measured following 3 h of mock, auxin, or brassinosteroid (BR) treatment. As seen in the T1 screen, loss of A2, A3, and A4 [*pS15-2E(ΔA4A3A2)*] or A2 alone [*pS15-2E(ΔA2)*] eliminated auxin and brassinosteroid response. Loss of E1 alone [*pS15-2E(ΔE1)*] or in combination with a loss in E2 [*pS15-2E(ΔE2E1)*] led to a similarly severe loss of reporter sensitivity to auxin and brassinosteroid treatment. Asterisks indicate significant differences in hormone response from *pS15-2E(ii)* identified using a two-way ANOVA followed by a Tukey's honestly significant differences test. Two biological replicates were assayed for each T2 line under each condition. Arrowheads indicate location of mutated elements. Error bars represent SE. **C**, Brassinosteroids and auxin can regulate MONOPTEROS/ARF5 and BES1 binding to DNA. ChIP assays were used to test candidate transcription factor binding to the region of the *SAUR15* promoter containing the essential A2 and E1 elements. Protein binding was assayed following 3 h of mock, auxin, or BR treatments. Binding of BES1:GFP to the *SAUR15* promoter could be detected in mock treated seedlings but was clearly enhanced by both hormone treatments. Similarly, MP:GFP binding was enhanced by treatment with either auxin or brassinosteroids. Results are expressed as a ratio of experimental condition to no antibody control, normalized to binding within the *SAUR15* open reading frame. Each treatment is the average of at least two independent biological replicates. Position of the PCR amplicons is indicated on schematic. Error bars represent SE. **D**, E1 is required for brassinosteroid enhancement of MP/ARF5 binding to DNA. ChIP assays were used to test MP:GFP binding to transgene promoters containing intact or mutated E1 sites. Results from two independent biological replicates are expressed as in C. Error bars represent SE. [See online article for color version of this figure.]

sponse. Auxin treatment was also able to increase BES1 binding, raising the possibility of a larger transcriptional complex. Such a model is complementary to the recent finding that auxin induces expression of a brassinosteroid biosynthetic enzyme through inhibition of BZR1 binding to its promoter (Chung et al., 2011).

Analysis of MP/ARF5 provided further evidence for a larger transcriptional complex representing factors regulated by multiple pathways. Previous studies suggested that MP/ARF5 regulates *SAUR15* expression (Schlereth et al., 2010) and can bind to A2-like elements (Donner et al., 2009). We performed ChIP assays on *pMP::MP::GFP* seedlings using primers flanking the critical minimal region where we observed BES1 binding. Although we found little evidence of MP/ARF5 binding in mock-treated samples, binding was clearly enriched following treatment with either auxin or brassinosteroids (Fig. 3C). Although no direct interaction has been shown between BES1 and any of the ARFs, other proteins may be acting as bridges between the two factors. If BES1 was required for brassinosteroid enhancement of MP/ARF5 binding, then we reasoned that loss of the E1 site should reduce the effects of brassinosteroids on MP/ARF5. To test this, we crossed *pMP::MP::GFP* plants to both *pS15-2E(ii)::LUC* and *pS15-2E(ΔE1)::LUC* plants. ChIP assays with transgene-specific primers showed brassinosteroid-induced increase in MP/ARF5 binding did largely depend on an intact E1 site (Fig. 3D).

Conservation of Critical cis-Regulatory Elements

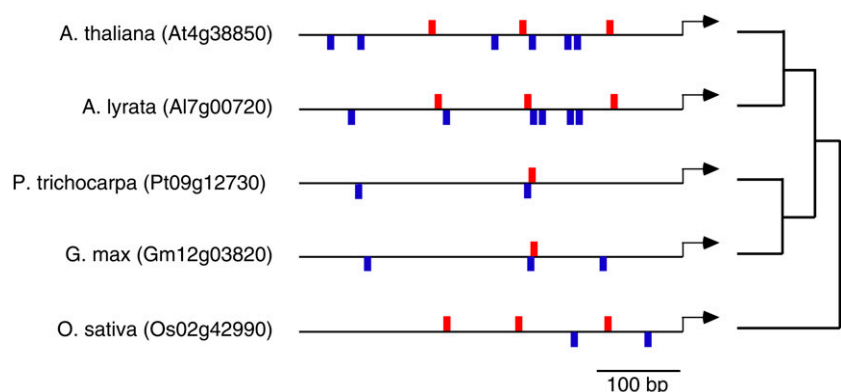
SAURs were originally identified in soybean (McClure et al., 1989) and can be identified in the genomes of many flowering plants. If the elements identified in this study are important for gene function, they would likely be preserved during species divergence. To test this prediction, we compared upstream regulatory regions of putative *SAUR15* orthologs in a number of species at increasing evolutionary distance from Arabidopsis. These included the close relative *Arabidopsis lyrata*, poplar (*Populus* spp.), soybean (*Glycine max*), and rice (*Oryza sativa*). This group spans more than 100 million years of evolutionary

time (Reineke et al., 2011). Although blocks of extended shared sequence were still readily detectable in *A. lyrata*, this was not the case with any of the other promoters.

Within 250 bases upstream of the predicted transcriptional start sites of every putative ortholog, we were able to identify HUD elements (CACATG/CATGTG; Fig. 4). Of all possible 5-mers contained within the 250 upstream bases, only four sequences were exactly conserved across all six genes examined here (AGACA, TCATG, TTCTC, TTTCT). This is consistent with a recent bioinformatic analysis showing rare conservation of plant promoter sequences across the monocot-dicot divide (Reineke et al., 2011). One of the 5-mers that was absolutely conserved was the A2-type of AuxRE variant (AGACA). In fact, at least one AGACA element was found within 50 bp of a HUD element in every promoter examined here.

To further examine the possible significance of clustering of elements, we analyzed promoters of all protein-coding genes in the Arabidopsis genome (27,206). We used only the first 250 bp of promoter sequence to more closely match the architecture of key *SAUR15* regulatory sequences. Under these conditions, approximately 10% (2818) of all promoters contained at least one HUD element, whereas more than 40% (11,055) of all promoters had at least one TGTCT/AGACA A2-like element. Less than 5% (1,222) of Arabidopsis promoters had both elements. We then took the list of all promoters containing one of the elements (e.g. HUD) and tested whether the total count of the other element (e.g. A2) was enriched when compared with promoters lacking the first element. Whether we tested for HUD element frequency in A2-containing promoters or for A2 element frequency in HUD-containing promoters, we detected small but statistically significant ($P < 0.0001$) increases in the rates of element co-occurrence. For example, the average rate of finding an A2 element increased from 0.54 in non-HUD promoters to 0.60 in HUD-containing promoters. This means that for every 100 randomly selected HUD-containing promoters, approximately six more A2 elements would be expected than in 100 randomly selected non-HUD containing promoters. Among the genes with both elements (Supplemental Table S1), there are a number of genes

Figure 4. HUD elements and A2-like elements are conserved across flowering plant species. Promoters of putative *SAUR15* orthologs show conservation of HUD (CACATG/CATGTG) and A2 (TGTCT/AGACA) elements close to transcriptional start sites. Regions 500 bp upstream of each ortholog are shown. Across all putative orthologs, HUD elements were found within 250 bp of transcriptional start site. At least one A2-type element could be identified within 50 bp of the HUD element. Red bars represent HUD elements and blue bars represent A2-like elements. [See online article for color version of this figure.]



affecting auxin biosynthesis (TSB1, TSB2, YUC2, YUC4, YUC9, CYP79F1/BUS1/SPS1, GH3.2/YDK1, GH3.3, GH3.10, GH3.17), transport (WAG1, PID2), and signaling (TIR1, AFB1, IAA6, IAA7, IAA17, IAA19, IAA32, ARF4, ARF16, SAUR19, SAUR50, SAUR62, SAUR63, SAUR64, SAUR65, SAUR66, SAUR67, SAUR68, SAR1, HAT2); brassinosteroid biosynthesis (CYP72C1/SOB7/SHK1, CYP710A1, CYP90D1) and signaling (BES1, PP2A2, BEE2); and cellular expansion (XTH17, XTH18, XTH19, XTH26, XTH33, EXPA11, EXPA13, PERK8, SAB, CESA8, CSLA14, CSLB01, CSLC5, CSLC6, AQP1).

Given the small number of elements found to be required for hormone response in the *SAUR15* promoter, it is not clear how many of the elements identified in this bioinformatic analysis are functionally relevant. One way to enrich for functionality is to focus on genes induced by auxin or brassinosteroids. Among stringently identified auxin-induced genes, nearly 14% (27 of 198) have both a HUD and an A2-like element within 250 bp of their predicted transcriptional start site; a similar percentage of brassinosteroid-induced genes exhibit this architecture (four of 28). In contrast, only one-half as many cytokinin-induced genes meet this criterion (four of 60). Of the 31 auxin- or brassinosteroid-induced genes with putative bipartite promoter elements (Supplemental Table S2), >60% (19) were identified as BES1 or BZR1 targets in global ChIP studies as compared with <17% of the whole genome. The results are similarly striking when the list of genes is compared with stringently identified MP targets: 20% (six) are likely bound by MP, compared with 0.4% of the genome as a whole.

DISCUSSION

Genome-wide surveys and computational analyses of auxin and brassinosteroid targets have provided clues into the regulatory landscape required for hormone response (Nemhauser et al., 2004; Priest et al., 2009; Keilwagen et al., 2011; Yamamoto et al., 2011). However, these approaches have a limited ability to define the function of specific cis-elements. Using the promoter region of *SAUR15* as a test case, we identified a short region sufficient for both auxin and brassinosteroid response [Fig. 3B; *pS15-1E(i)::LUC*]. A detailed analysis of specific cis-elements revealed two critical elements coregulated by both hormones: an AuxRE variant (TGTCTG/CAGACA) and a HUD-type E-box (CACATG/CATGTG). Disruption of either of these cis-regulatory elements resulted in a loss of hormone response.

In this work, we found that despite apparent complexity in quantitative responses to hormone treatment, there is an absolute need for two elements: one predicted to be regulated by auxin and the other by brassinosteroids. This binary requirement for each pathway to be present to allow response to the other can be compared to an AND logic gate. The use of AND logic can lead to more robust pathway function because cells need to receive sustained signals from

both pathways to activate gene expression. A recent global transcriptome analysis of the effect of the brassinosteroid biosynthetic inhibitor brassinazole on auxin response showed a striking reduction of auxin response when brassinosteroid levels were compromised (Chung et al., 2011). Enhancement of BES1 and MP/ARF5 binding with application of either hormone (Fig. 3C), combined with evidence that brassinosteroid enhancement of MP/ARF5 requires an intact E-box (Fig. 3D), make a strong case that interdependence of auxin and brassinosteroid signaling reflects joint regulation of promoter activation. Full activation of BES1 and MP/ARF5 require additional pathway-specific events, which could be facilitated by preformation of a poised complex bound to DNA.

Recent evidence of BES1 interaction partners may provide a clue to why auxin response requires the E1 site. BES1 has been shown to interact with proteins that promote transcriptional elongation (Li et al., 2010). In this way, BES1 may be needed to globally prime growth-associated targets by promoting more efficient transcription. Interestingly, the critical mammalian bHLH transcription factor c-Myc may have a parallel function. A recent study of c-Myc targets showed enrichment of E-boxes within 200 bp of the transcriptional start site specifically among genes associated with paused RNA Pol II (Rahl et al., 2010). Interaction with RNA Pol II may also explain why even in plant species with larger average intergenic regions, HUD-type E-boxes and A2-like elements were still found close to transcriptional start sites in putative *SAUR15* orthologs (Fig. 4). A model where BES1 acts to boost response to various stimuli is consistent with the role of brassinosteroids in auxin response analyzed here and the observed role of brassinosteroids in enhancing differential cell fate determination during root epidermal development (Kuppusamy et al., 2009).

Our analysis of putative *SAUR15* orthologs promoter regions revealed conservation specifically of HUD type E-boxes. HUD elements are overrepresented in phytohormone gene promoters and are believed to play a critical role in the temporal syncing of growth-related processes (Michael et al., 2008). Expression of a number of key brassinosteroid biosynthesis and signaling genes show diel cycling (Bancos et al., 2006; Michael et al., 2008). In addition, many genes related to auxin metabolism and response are regulated by the clock (Covington and Harmer, 2007). Clustering of HUD and A2-like elements could facilitate coordination of auxin and brassinosteroid responses to specific times of day.

Auxin promotion of BES1 binding was an unexpected result (Fig. 4), particularly given previous evidence that auxin treatment does not affect the phosphorylation status of BES1 (Yin et al., 2002), and BES1 needs to be dephosphorylated to bind DNA (Vert and Chory, 2006). Phosphorylation of ARF2 by the brassinosteroid-regulated kinase BIN2 was shown to reduce ARF2 binding to DNA (Vert et al., 2008), but little is known about whether posttranslational mod-

ifications play a major role in regulating ARF-DNA associations. Each hormone could influence binding of the other pathway's transcription factor through regulation of cofactors. For example, MYB77 has been shown to interact with ARF7 (Shin et al., 2007) and MYB30 has been shown to directly interact with BES1 (Li et al., 2009). An alternative explanation is that each hormone is affecting the level of the other. An auxin-induced rise in brassinosteroid levels might be sufficient to trigger the modest increase in binding we observed, yet not sufficiently change phosphorylation status to be detected by western blots. *BREVIS RADIX* is known to act in just such a secondary cross-regulation loop (Mouchel et al., 2006), linking auxin perception with increased expression of a key enzyme in brassinosteroid biosynthesis. Brassinosteroids could also cause a rise in auxin levels, though for the timelines used here that is less likely. Studies of the auxin-labile Aux/IAAs (Zenser, et al., 2003) suggest that auxin levels are not significantly affected by brassinosteroid treatment.

Another question raised by our results is why the widely used DR5 promoter, which consists only of multimerized TGTCTCs, is responsive to both auxin and brassinosteroids (Nakamura et al., 2003b; Nemhauser et al., 2004). One possible explanation is that the number of AuxRE repeats in the synthetic promoter may bypass the need for whatever function is provided here by the E-box. For example, there may be a change in chromatin structure or strength of induction when multiple ARFs can bind to the same promoter. It is worth noting that few natural promoters show extensive AuxRE arrays. Another interesting possibility is that different ARFs have different requirements for full activation. The *SAUR15* promoter examined here was analyzed only in seedlings. There have been reports of different relationships between auxin and brassinosteroids in other cell types, such as mesophyll protoplasts (Wang et al., 2005). These differences could be explained by which ARFs or other potential partner proteins are available in specific environments and whether brassinosteroids have any effect on those partners. Brassinosteroids have been shown to induce expression of some ARFs and a number of Aux/IAA corepressors (Goda et al., 2004; Nemhauser et al., 2004; Kim et al., 2006). They have also been implicated in inhibition of DNA binding by repressor ARFs (Vert et al., 2008). These mechanisms could contribute to the brassinosteroid effect on isolated AuxREs, as well as those found in native promoters.

Global transcriptome experiments suggest that there may be multiple classes of auxin- and brassinosteroid-responsive genes (Goda et al., 2004; Nemhauser et al., 2004, 2006), a conclusion supported by this study. The bipartite element identified here likely identifies one class of genes with a distinct mode of auxin:brassinosteroid cross-regulation. By analyzing the functional requirements of elements found in other promoters, further groupings may be found. Investigation of other

promoters with predicted bipartite elements, across diverse tissues and under different environmental conditions, is needed to test whether shared architecture leads to a functional module of coregulation. In particular, using genes with a stronger hormone induction than what was observed here for *SAUR15* could provide important additional information. This type of directed functional analysis of response elements is essential for "ground-truthing" global data mining efforts and for ultimately understanding gene network dynamics.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis (*Arabidopsis thaliana*) ecotype Columbia was used as wild type throughout this study. Several constructs were previously characterized and generously provided by others (*SAUR-AC1::GUS*, Dr. Pamela Green [University of Delaware]; *pMP::MP::GFP* seeds, Dr. Dolf Weijers [Wageningen University]; and *pBES1::BES1::GFP* seeds, Dr. Zhi-Yong Wang [Standard University]). Seeds were surface sterilized for 15 min in 70% ethanol with 0.05% Triton X-100 followed by 5 min in 95% ethanol. After sterilization, seeds were spread onto plates containing 0.5× Linsmaier and Skoog (LS) media (Caisson Laboratories, Inc.) with 0.8% agar and stratified in the dark at 4°C for 2 d. All seedlings were 9 d old at the time of the assays. Seedlings were grown at 20°C in 12-h-light/12-h-dark conditions in 35 $\mu\text{mol m}^{-2} \text{s}^{-1}$ white light, except for T1 seedlings, which were grown in 80 $\mu\text{mol m}^{-2} \text{s}^{-1}$ white light. Plants were transformed by the floral dip method (Clough and Bent, 1998), and transgenic lines were identified by selection on kanamycin (Fisher Scientific).

Quantitative RT-PCR

Light-grown seedlings were immersed in 0.5× LS media (Caisson Laboratories, Inc.) containing mock (0.1% dimethyl sulfoxide; Sigma), 1 μM brassinolide (Chemiclones), or 1 μM auxin (IAA; Bioworld) treatments for 1 and 3 h. Total RNA was extracted by using an RNeasy kit (Qiagen), and first strand cDNA was synthesized by using an iScript cDNA Synthesis kit (Bio-Rad). cDNAs were diluted 20-fold and combined with SYBR master mix (Bio-Rad). Three independent biological replicates were analyzed with a Chromo4 Real-Time PCR machine (MJ Research). Expression for each gene was calculated using the formula $(E_{\text{target}})^{-\Delta\text{Ct}_{\text{target}}(\text{control} - \text{sample})} / (E_{\text{ref}})^{-\Delta\text{Ct}_{\text{ref}}(\text{control} - \text{sample})}$ (Pfaffl, 2001) and normalized to a reference gene, *At1g13320*.

Plasmid Construction

All deletion constructs were generated by PCR amplification within the intergenic region between *At4g38840* and *At4g38850* (*SAUR15*) from wild-type genomic DNA. Amplified regions were inserted into a pCR-BLUNT vector (Invitrogen), then digested and ligated into pGreen I 0029 (Hellens et al., 2000) containing the LUC+ reporter. Phusion site-directed mutagenesis kit (New England BioLabs) was used to mutate transcriptional elements in *pS15-2E(ii)*. Primers for deletion constructs and site-directed mutagenesis are listed in Supplemental Table S3.

Luciferase Analysis

Seedlings growing on plates were presprayed 16 h prior to assay with 2.5 mM D-luciferin (Biosynth) and 0.01% Triton X-100. At the beginning of the hormone analysis, whole seedlings were transferred from agar plates to 96-well Optiplates (PerkinElmer) containing 0.5× LS media. Luciferin and either hormone or mock treatments were subsequently added to each well. Luciferase activity was measured using a Victor V³ Plate Reader (PerkinElmer). Baseline measurements of luciferase activity for T1 seedlings were taken after 20 min to allow for autoluminescence to decay (measured by examining seedlings lacking reporter constructs). Hormone responses were measured at 3 h. For experiments with T1s, ratios of luciferase activity at 3 h versus baseline were calculated for each individual line. Linear regression was used to test for

significant differences in auxin response between constructs. For T2 experiments, significant differences in auxin or brassinosteroid response between lines were identified using a two-way ANOVA followed by a Tukey's honestly significant difference test.

GUS Staining

The GUS staining protocol was as described by Sessions et al. (1999). In brief, seedlings were incubated in staining solution [50 mM NaPO₄, 0.2% Triton X-100, 10 mM K₄Fe(CN₆)-3H₂O, 10 mM K₃Fe(CN₆), and 1 mM 5-bromo-4-chloro-3-indolyl-β-D-GlcA (X-gluc; Rose Scientific, LTD.)] for 5 h at 37°C following vacuum infiltration and then fixed in 50% ethanol, 3.7% formaldehyde, and 5% acetic acid.

ChIP Assays

Nine-day-old seedlings (*pBES1::BES1::GFP* and *pMP::MP::GFP*) were cross-linked in 1% formaldehyde under vacuum. 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 MgCl₂, 5% Dextran T-40, 2.5% Ficoll, 20 mM β-mercaptoethanol, and mini-Complete Proteinase Inhibitor tablet (Roche Applied Science)], filtered through Miracloth (Fisher Scientific), 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 a Fisher Dismembrator (Fisher Scientific). An aliquot of fragmented chromatin served as an input control for qPCR analysis, and the remainder was subjected to immunoprecipitation. Dynabeads Protein A (Invitrogen) coupled with anti-GFP (Ab290, Abcam) antibody were used to enrich for pMP::MP::GFP- or pBES1::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 (Qiagen). ChIP assays were normalized to a region in the *ACT2* 3' untranslated region, and results are expressed as ratios of qPCR signal to the no antibody IP. ChIP results represent the average of at least two independent biological replicates normalized to the region designated ORF in *SAUR15* open reading frame (Fig. 3C). Primers for ChIP qPCR analysis are listed in Supplemental Table S4.

Phylogenetic Tree and Promoter Analysis

Putative orthologs for *SAUR15* were identified using the "Peptide Homologs" function of Phytozome v.5.0 (<http://www.phytozome.net/>) and the "orthologs of other genes" function of PLAZA database (Proost et al., 2009). Five hundred bp of sequence 5' of the coding region was analyzed for E-boxes and TGCT-like elements. NJplot software (<http://pbil.univ-lyon1.fr/software/njplot.html>) was used to construct an unrooted phylogenetic tree for aligned coding sequences of putative *SAUR15* orthologs using the neighbor-joining method. Multiple alignments of coding sequences were performed using ClustalX (<http://www.ebi.ac.uk/Tools/msa/clustalw2/>). Conservation of possible 5-mers were calculated using a custom script in MATLAB (MathWorks). Whole-genome analysis was based on TAIR10 annotations (<http://arabidopsis.org>). Custom scripts (perl, <http://www.perl.org/>) were used to extract the first 250 bp of upstream regulatory regions for all protein-coding genes and count occurrences of HUD and A2-type elements. Element distributions were compared in R (<http://www.r-project.org/>) using a general linear model given a Poisson distribution. Promoters were divided into those containing a specific element or not (HUD-containing, non-HUD; A2-containing, non-A2). This categorization was used to determine if the occurrence of one element was a significant factor in determining the likelihood of finding the other element. In both cases (frequency of HUD elements in A2-containing promoters and frequency of A2 elements in HUD-containing promoters), there was a positive, highly significant coefficient ($P < 0.0001$). All scripts are available upon request.

Supplemental Data

The following materials are available in the online version of this article.

Supplemental Figure S1. Baseline expression levels of reporter constructs.

Supplemental Table S1. Arabidopsis genes with both HUD and A2-type elements.

Supplemental Table S2. Auxin- and/or brassinosteroid-induced genes with HUD and A2-type elements.

Supplemental Table S3. Primers for deletion and mutation constructs.

Supplemental Table S4. Primers for ChIP assays.

ACKNOWLEDGMENTS

We thank Pam Green, Dolf Weijers, and Zhi-Yong Wang for generous gifts of seeds and protocols; Eva Farre for guidance on seedling luciferase assays; Jodi Stewart, Janneke Hille Ris Lambers, and Yaowu Yuan for assistance with promoter analysis; Roger Deal for assistance with ChIP assays; Anahit Galstyan, Andrew Chen, Edison Calaunan, Rachel Denney, Zachary McCauley, and Autumn Walker for technical assistance; Amanda Rychel for assistance with the phylogenetic analysis; and Keiko Torii, David Parichy, Takato Imaizumi, Elizabeth Van Volkenburgh, Stephanie Busch, Larissa Patterson, Jodi Stewart, John Chevillet, Jennifer Lachowicz, Christopher Gee, and Jessica Guseman for excellent discussions and critical evaluation of this manuscript.

Received September 19, 2011; accepted November 16, 2011; published November 18, 2011.

LITERATURE CITED

- Bancos S, Szatmári AM, Castle J, Kozma-Bognár L, Shibata K, Yokota T, Bishop GJ, Nagy F, Szekeres M (2006) Diurnal regulation of the brassinosteroid-biosynthetic CPD gene in Arabidopsis. *Plant Physiol* **141**: 299–309
- Bao F, Shen J, Brady SR, Muday GK, Asami T, Yang Z (2004) Brassinosteroids interact with auxin to promote lateral root development in Arabidopsis. *Plant Physiol* **134**: 1624–1631
- Beuchat J, Scacchi E, Tarkowska D, Ragni L, Strnad M, Hardtke CS (2010) BRX promotes Arabidopsis shoot growth. *New Phytol* **188**: 23–29.
- Chandler JW, Cole M, Flier A, Werr W (2009) BIM1, a bHLH protein involved in brassinosteroid signalling, controls Arabidopsis embryonic patterning via interaction with DORNROSCHEN and DORNROSCHEN-LIKE. *Plant Mol Biol* **69**: 57–68
- Chapman EJ, Estelle M (2009) Mechanism of auxin-regulated gene expression in plants. *Annu Rev Genet* **43**: 265–285
- Chung Y, Maharjan PM, Lee O, Fujioka S, Jang S, Kim B, Takatsuto S, Tsujimoto M, Kim H, Cho S, et al (2011) Auxin stimulates DWARF4 expression and brassinosteroid biosynthesis in Arabidopsis. *Plant J* **66**: 564–578
- Clough SJ, Bent AF (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J* **16**: 735–743
- Cole M, Chandler J, Weijers D, Jacobs B, Comelli P, Werr W (2009) DORNROSCHEN is a direct target of the auxin response factor MONOPTEROS in the Arabidopsis embryo. *Development* **136**: 1643–1651
- Covington ME, Harmer SL (2007) The circadian clock regulates auxin signaling and responses in Arabidopsis. *PLoS Biol* **5**: e222
- Donner TJ, Sherr I, Scarpella E (2009) Regulation of preprocambial cell state acquisition by auxin signaling in Arabidopsis leaves. *Development* **136**: 3235–3246
- Gil P, Green PJ (1996) Multiple regions of the Arabidopsis SAUR-AC1 gene control transcript abundance: the 3' untranslated region functions as an mRNA instability determinant. *EMBO J* **15**: 1678–1686
- Gil P, Green PJ (1997) Regulatory activity exerted by the SAUR-AC1 promoter region in transgenic plants. *Plant Mol Biol* **34**: 803–808
- Gil P, Liu Y, Orbović V, Verkamp E, Poff KL, Green PJ (1994) Characterization of the auxin-inducible SAUR-AC1 gene for use as a molecular genetic tool in Arabidopsis. *Plant Physiol* **104**: 777–784
- Goda H, Sawa S, Asami T, Fujioka S, Shimada Y, Yoshida S (2004) Comprehensive comparison of auxin-regulated and brassinosteroid-regulated genes in Arabidopsis. *Plant Physiol* **134**: 1555–1573
- Goda H, Shimada Y, Asami T, Fujioka S, Yoshida S (2002) Microarray analysis of brassinosteroid-regulated genes in Arabidopsis. *Plant Physiol* **130**: 1319–1334
- Gray WM, del Pozo JC, Walker L, Hobbie L, Risseuw E, Banks T, Crosby WL, Yang M, Ma H, Estelle M (1999) Identification of an SCF ubiquitin-ligase complex required for auxin response in Arabidopsis thaliana. *Genes Dev* **13**: 1678–1691
- Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCF(TIR1)-dependent degradation of AUX/IAA proteins. *Nature* **414**: 271–276

- Guilfoyle TJ, Hagen G** (2007) Auxin response factors. *Curr Opin Plant Biol* **10**: 453–460
- Hardtke CS** (2007) Transcriptional auxin-brassinosteroid crosstalk: who's talking? *Bioessays* **29**: 1115–1123
- Hardtke CS, Dorcey E, Osmont KS, Sibout R** (2007) Phytohormone collaboration: zooming in on auxin-brassinosteroid interactions. *Trends Cell Biol* **17**: 485–492
- He JX, Gendron JM, Sun Y, Gampala SS, Gendron N, Sun CQ, Wang ZY** (2005) BZR1 is a transcriptional repressor with dual roles in brassinosteroid homeostasis and growth responses. *Science* **307**: 1634–1638
- Hellens RP, Edwards EA, Leyland NR, Bean S, Mullineaux PM** (2000) pGreen: a versatile and flexible binary Ti vector for Agrobacterium-mediated plant transformation. *Plant Mol Biol* **42**: 819–832
- Higo K, Ugawa Y, Iwamoto M, Korenaga T** (1999) Plant cis-acting regulatory DNA elements (PLACE) database: 1999. *Nucleic Acids Res* **27**: 297–300
- Keilwagen J, Grau J, Paponov IA, Posch S, Strickert M, Grosse I** (2011) De-novo discovery of differentially abundant transcription factor binding sites including their positional preference. *PLoS Comput Biol* **7**: e1001070
- Kim H, Park PJ, Hwang HJ, Lee SY, Oh MH, Kim SG** (2006) Brassinosteroid signals control expression of the AXR3/IAA17 gene in the crosstalk point with auxin in root development. *Biosci Biotechnol Biochem* **70**: 768–773
- Kim TW, Guan S, Sun Y, Deng Z, Tang W, Shang JX, Sun Y, Burlingame AL, Wang ZY** (2009) Brassinosteroid signal transduction from cell-surface receptor kinases to nuclear transcription factors. *Nat Cell Biol* **11**: 1254–1260
- Kim TW, Wang ZY** (2010) Brassinosteroid signal transduction from receptor kinases to transcription factors. *Annu Rev Plant Biol* **61**: 681–704
- Kuppusamy KT, Chen AY, Nemhauser JL** (2009) Steroids are required for epidermal cell fate establishment in Arabidopsis roots. *Proc Natl Acad Sci USA* **106**: 8073–8076
- Li L, Xu J, Xu ZH, Xue HW** (2005) Brassinosteroids stimulate plant tropisms through modulation of polar auxin transport in Brassica and Arabidopsis. *Plant Cell* **17**: 2738–2753
- Li L, Ye H, Guo H, Yin Y** (2010) Arabidopsis IWS1 interacts with transcription factor BES1 and is involved in plant steroid hormone brassinosteroid regulated gene expression. *Proc Natl Acad Sci USA* **107**: 3918–3923
- Li L, Yu X, Thompson A, Guo M, Yoshida S, Asami T, Chory J, Yin Y** (2009) Arabidopsis MYB30 is a direct target of BES1 and cooperates with BES1 to regulate brassinosteroid-induced gene expression. *Plant J* **58**: 275–286
- McClure BA, Hagen G, Brown CS, Gee MA, Guilfoyle TJ** (1989) Transcription, organization, and sequence of an auxin-regulated gene cluster in soybean. *Plant Cell* **1**: 229–239
- Michael TP, Mockler TC, Breton G, McEntee C, Byer A, Trout JD, Hazen SP, Shen R, Priest HD, Sullivan CM, et al** (2008) Network discovery pipeline elucidates conserved time-of-day-specific cis-regulatory modules. *PLoS Genet* **4**: e14
- Mouchel CF, Osmont KS, Hardtke CS** (2006) BRX mediates feedback between brassinosteroid levels and auxin signalling in root growth. *Nature* **443**: 458–461
- Müssig C, Fischer S, Altmann T** (2002) Brassinosteroid-regulated gene expression. *Plant Physiol* **129**: 1241–1251
- Nakamura A, Higuchi K, Goda H, Fujiwara MT, Sawa S, Koshiba T, Shimada Y, Yoshida S** (2003a) Brassinolide induces IAA5, IAA19, and DR5, a synthetic auxin response element in Arabidopsis, implying a cross talk point of brassinosteroid and auxin signaling. *Plant Physiol* **133**: 1843–1853
- Nakamura A, Shimada Y, Goda H, Fujiwara MT, Asami T, Yoshida S** (2003b) AXR1 is involved in BR-mediated elongation and SAUR-AC1 gene expression in Arabidopsis. *FEBS Lett* **553**: 28–32
- Nakamura A, Nakajima N, Goda H, Shimada Y, Hayashi K, Nozaki H, Asami T, Yoshida S, Fujioka S** (2006) Arabidopsis Aux/IAA genes are involved in brassinosteroid-mediated growth responses in a manner dependent on organ type. *Plant J* **45**: 193–205
- Nemhauser JL, Hong F, Chory J** (2006) Different plant hormones regulate similar processes through largely nonoverlapping transcriptional responses. *Cell* **126**: 467–475
- Nemhauser JL, Mockler TC, Chory J** (2004) Interdependency of brassinosteroid and auxin signaling in Arabidopsis. *PLoS Biol* **2**: E258
- Pfaffl MW** (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* **29**: e45
- Priest HD, Filichkin SA, Mockler TC** (2009) Cis-regulatory elements in plant cell signaling. *Curr Opin Plant Biol* **12**: 643–649
- Proost S, Van Bel M, Sterck L, Billiau K, Van Parys T, Van de Peer Y, Vandepoel K** (2009) PLAZA: a comparative genomics resource to study gene and genome evolution in plants. *Plant Cell* **21**: 3718–3731
- Rahl PB, Lin CY, Seila AC, Flynn RA, McCuine S, Burge CB, Sharp PA, Young RA** (2010) c-Myc regulates transcriptional pause release. *Cell* **141**: 432–445
- Reineke AR, Bornberg-Bauer E, Gu J** (2011) Evolutionary divergence and limits of conserved non-coding sequence detection in plant genomes. *Nucleic Acids Res* **39**: 6029–6043
- Schlereth A, Möller B, Liu W, Kientz M, Flipse J, Rademacher EH, Schmid M, Jürgens G, Weijers D** (2010) MONOPTEROS controls embryonic root initiation by regulating a mobile transcription factor. *Nature* **464**: 913–916
- Sessions A, Weigel D, Yanofsky MF** (1999) The Arabidopsis thaliana MERISTEM LAYER 1 promoter specifies epidermal expression in meristems and young primordia. *Plant J* **20**: 259–263
- Shin R, Burch AY, Huppert KA, Tiwari SB, Murphy AS, Guilfoyle TJ, Schachtman DP** (2007) The Arabidopsis transcription factor MYB77 modulates auxin signal transduction. *Plant Cell* **19**: 2440–2453
- Stewart JL, Nemhauser JL** (2010) Do trees grow on money? Auxin as the currency of the cellular economy. *Cold Spring Harb Perspect Biol* **2**: a001420
- Sun Y, Fan XY, Cao DM, Tang W, He K, Zhu JY, He JX, Bai MY, Zhu S, Oh E, et al** (2010) Integration of brassinosteroid signal transduction with the transcription network for plant growth regulation in Arabidopsis. *Dev Cell* **19**: 765–777
- Szemenyei H, Hannon M, Long JA** (2008) TOPLESS mediates auxin-dependent transcriptional repression during Arabidopsis embryogenesis. *Science* **319**: 1384–1386
- Tiwari SB, Wang XJ, Hagen G, Guilfoyle TJ** (2001) AUX/IAA proteins are active repressors, and their stability and activity are modulated by auxin. *Plant Cell* **13**: 2809–2822
- Ulmasov T, Liu ZB, Hagen G, Guilfoyle TJ** (1995) Composite structure of auxin response elements. *Plant Cell* **7**: 1611–1623
- Vert G, Chory J** (2006) Downstream nuclear events in brassinosteroid signalling. *Nature* **441**: 96–100
- Vert G, Walcher CL, Chory J, Nemhauser JL** (2008) Integration of auxin and brassinosteroid pathways by Auxin Response Factor 2. *Proc Natl Acad Sci USA* **105**: 9829–9834
- Wang S, Tiwari SB, Hagen G, Guilfoyle TJ** (2005) AUXIN RESPONSE FACTOR7 restores the expression of auxin-responsive genes in mutant Arabidopsis leaf mesophyll protoplasts. *Plant Cell* **17**: 1979–1993
- Wang ZY, Nakano T, Gendron J, He J, Chen M, Vafeados D, Yang Y, Fujioka S, Yoshida S, Asami T, Chory J** (2002) Nuclear-localized BZR1 mediates brassinosteroid-induced growth and feedback suppression of brassinosteroid biosynthesis. *Dev Cell* **2**: 505–513
- Xu N, Hagen G, Guilfoyle T** (1997) Multiple auxin response modules in the soybean SAUR 15A promoter. *Plant Sci* **126**: 193–201
- Yamamoto YY, Yoshioka Y, Hyakumachi M, Maruyama K, Yamaguchi-Shinozaki K, Tokizawa M, Koyama H** (2011) Prediction of transcriptional regulatory elements for plant hormone responses based on microarray data. *BMC Plant Biol* **11**: 39
- Yin Y, Vafeados D, Tao Y, Yoshida S, Asami T, Chory J** (2005) A new class of transcription factors mediates brassinosteroid-regulated gene expression in Arabidopsis. *Cell* **120**: 249–259
- Yin Y, Wang ZY, Mora-Garcia S, Li J, Yoshida S, Asami T, Chory J** (2002) BES1 accumulates in the nucleus in response to brassinosteroids to regulate gene expression and promote stem elongation. *Cell* **109**: 181–191
- Yu X, Li L, Zola J, Aluru M, Ye H, Foudree A, Guo H, Anderson S, Aluru S, Liu P, Roderick S, Yin Y** (2011) A brassinosteroid transcriptional network revealed by genome-wide identification of BES1 target genes in Arabidopsis thaliana. *Plant J* **65**: 634–646
- Zenser N, Dreher KA, Edwards SR, Callis J** (2003) Acceleration of Aux/IAA proteolysis is specific for auxin and independent of AXR1. *Plant J* **35**: 285–294
- Zhang S, Cai Z, Wang X** (2009) The primary signaling outputs of brassinosteroids are regulated by abscisic acid signaling. *Proc Natl Acad Sci USA* **106**: 4543–4548