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FRUITFULL controls SAUR10 expression and regulates Arabidopsis growth and architecture

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Abstract

MADS-domain transcription factors are well known for their roles in plant development and regulate sets of downstream genes that have been uncovered by high-throughput analyses. A considerable number of these targets are predicted to function in hormone responses or responses to environmental stimuli, suggesting that there is a close link between developmental and environmental regulators of plant growth and development. Here, we show that the Arabidopsis MADS-domain factor FRUITFULL (FUL) executes several functions in addition to its noted role in fruit development. Among the direct targets of FUL, we identified SMALL AUXIN UPREGULATED RNA 10 (SAUR10), a growth regulator that is highly induced by a combination of auxin and brassinosteroids and in response to reduced R:FR light. Interestingly, we discovered that SAUR10 is repressed by FUL in stems and inflorescence branches. SAUR10 is specifically expressed at the abaxial side of these branches and this localized activity is influenced by hormones, light conditions and by FUL, which has an effect on branch angle. Furthermore, we identified a number of other genes involved in hormone pathways and light signalling as direct targets of FUL in the stem, demonstrating a connection between developmentally and environmentally regulated growth programs.

Key words: Architecture, auxin, branching, FRUITFULL, growth, hormones, light response, MADS-box transcription factor, SAUR.

Introduction

Plant growth and development are regulated by interplay between internal and external factors. The timely expression of different sets of transcription factors regulates the default program of plant growth and development, but this program is highly influenced by external factors that allow the plant to adapt its growth according to the environmental conditions. As a result, plants with the same genotype show distinct phenotypic differences when, for example, grown at different temperatures or under different light conditions. This response to environmental conditions is mainly...
regulated via hormonal pathways and involves auxin, gibberellic acid (GA), cytokinin and brassinosteroids (BRs). In particular auxin, which induces cell elongation, has been shown to be essential for growth responses to environmental conditions such as phototropism and gravitropism (Paponov et al., 2008; Fankhauser and Christie, 2015). Recently, the light-regulated growth of Arabidopsis hypocotyls has been thoroughly investigated and revealed to depend on physical interactions between transcription factors involved in auxin, GA, BR and light responses (Bai et al., 2012; Oh et al., 2014; Ross and Quittenden, 2016). Downstream growth-regulating genes can be induced or repressed by either the hormone-mediated environmental response pathway or by the internal developmental pathway, thus integrating these two pathways in the growth response.

A group of growth regulators that has been shown to be highly responsive to auxin and other hormonal stimuli is the SAUR family of Small Auxin-Upregulated RNAs. SAUR transcripts were first discovered in soybean and found to be rapidly upregulated after addition of auxin. Additional research in soybean and other species has revealed that SAUR activity is highly dynamic, as both transcript and protein half-lives were reported to be extremely short (McClure and Guillfoyle, 1987; Knauss et al., 2003). In Arabidopsis, the SAUR gene family contains 79 genes (Ren and Gray, 2015), of which approximately two-thirds have been found to respond to auxin in certain tissues (Paponov et al., 2008; Chapman et al., 2012; Bargmann et al., 2013). In addition, several SAUR genes have been found to be influenced by other hormones like abscisic acid (ABA), ethylene, GA and BRs (Kodaira et al., 2011; Walcher and Nemhauser, 2012; Stamm and Kumar, 2013; Oh et al., 2014; Li et al., 2015). The function of several Arabidopsis SAUR genes has been investigated using overexpression studies, unveiling their general capacity to promote cell elongation in growth-related processes (Chae et al., 2012; Spartz et al., 2012; Stamm and Kumar, 2013; Ren and Gray, 2015; Sun et al., 2016). For a long time, it was unknown how induced SAUR gene expression could result in increased cell elongation, but a study by Spartz et al. (2014) recently unveiled that SAURs act according to the earlier postulated acid growth theory (Rayle and Cleland, 1992). They interact with protein phosphatases of the PP2C-D family to inhibit their function, thereby preventing dephosphorylation of plasma membrane H+·ATPases, resulting in activation of these membrane pumps. Activation of the H+·ATPases leads to membrane acidification, which enables cell elongation. Different Arabidopsis SAURs were tested and they were all able to interact with PP2C-Ds (Spartz et al., 2014; Sun et al., 2016).

In addition to being responsive to hormones, SAUR genes have also been reported as targets of several transcription factors involved in plant development, such as the MADS domain transcription factors SEPALATA3 (SEP3) and APETALA1 (API), and the TCP (TEOSINTE BRANCHED1/CYCLOIDEA/PROLIFERATING CELL FACTOR1) family protein TCP20 (Kaufmann et al., 2009; Kaufmann et al., 2010b; Danisman et al., 2012), suggesting that growth regulators of the SAUR family can act as integrators of the developmental and environmental growth pathways. However, the interaction between both pathways during plant growth is poorly understood.

We performed a ChIP-seq experiment with the Arabidopsis MADS-domain factor FRUITFULL (FUL) and identified two closely related SAUR genes, SAUR10 and SAUR16, as strongly bound target genes. FUL is a major player in the network that regulates Arabidopsis fruit development and determines both fruit patterning and growth (Gu et al., 1998; Ferrándiz et al., 2000b). In addition, ful mutants were also reported to flower later than wild-type (Ferrándiz et al., 2000a) and to exhibit an altered cauline leaf shape (Gu et al., 1998). Here, we demonstrate that FUL plays additional and novel roles in plant growth and is able to directly regulate genes involved in hormone- and light-induced cell elongation, such as the DELLA genes RGL2 and GAI, PHOTOTROPHIC INTERACTING FACTOR 3-LIKE 1 (PIL1), the CYTOKININ OXIDASES CKX5 and CKX6, and SAUR10. The architecture phenotype of ful mutants, which exhibit more vertical branch growth, can be explained by the de-repression of SAUR10, which is specifically expressed at the abaxial side of the branch. SAUR10 is repressed by FUL in the stem but can be highly induced by a combination of auxin and brassinosteroids and is upregulated by simulated shade. Both the activity of FUL and the light conditions influence the specific expression of SAUR10 in branches and thereby affects the Arabidopsis branch angle phenotype. SAUR10 is thus responsive to both developmental and environmental cues and integrates both in the growth response.

**Materials and methods**

**Plant materials and growth conditions**

Most plants used in this study were in the Col-0 background, including the overexpression and reporter lines and the ful-7 (SALK_033647) mutant. For the ChIP analysis, FUL-GFP lines were used from a mixed Ler (ful-1)/Col-0 background (Urbanus et al., 2009). The JIC SM T-DNA insertion line SM_3_1724 was received from NASC (Tissier et al., 1999) and the FLAG T-DNA line FLAG_S90D09 (Samson et al., 2002) was received from the JPB in Versailles. Plants were grown on rockwool blocks watered with HYPONeX® solution (1.5 g/l), in a long-day climate chamber (16 h/8 h) at 22°C. The climate chamber was equipped with LED lights, resulting in the following control conditions: 87.6 μmol m⁻² s⁻¹ photosynthetically active radiation (PAR); R:FR ratio=30.1. Reduced R:FR conditions were achieved by supplemental far red (730 nm) irradiation, resulting in a PAR of 83.5 μmol m⁻² s⁻¹, and a R:FR ratio of 1.15.

**ChIP-Seq analysis**

For ChIP-Seq analysis, pistils/siliques in stages 12–16 were harvested from gFUL-GFP lines (Urbanus et al., 2009). The ChIP-Seq and subsequent data analysis were performed according to Kaufmann et al. (2010a). Input samples were used as controls. The data analyses were largely performed as described by van Mourik et al. (2015). Sequences from each ChIP library were mapped to the unmasked Arabidopsis thaliana genome (TAIR9) using SOAPv2 (Li and Durbin, 2009). A maximum of two mismatches and no gaps were allowed. Only uniquely mapped reads were retained. Sequence reads mapping to the plastid and mitochondrial genomes were eliminated. The R package CSAR was used for peak calling (Muñoz et al.,...
The ChIP-seq data have been deposited at the NCBI Gene Expression Omnibus (GEO) under accession number GSE79554.

**Electrophoretic mobility shift assays (EMSAs)**

SAUR10 and SAUR16 coding sequences were amplified from wild-type Col-0 cDNA and cloned into pSPUTK (see Supplementary Table S2) at JXB online for all primer sequences. The pSPUTK promoter allowed in vitro protein synthesis using the TNT® SP6 High-Yield Wheat Germ Protein Expression System (Promega) according to manufacturers instructions. For SAUR10, the probe fragment consisted of a region of 100 bp with the canonical CArG box in the centre. For SAUR16, the fragment consisted of a region of 128 bp below the peak summit (see Supplementary Fig. S2 and Table S2 for primer sequences). Promoter fragments were amplified from genomic DNA; the complete FUL coding sequence was amplified from cDNA. The mutated SAUR10 fragment was generated by overlapping PCR using primers that replaced the canonical CCAATATG CG box with CCAACGATGG. EMSAs were performed essentially as described by Smaczniak et al. (2012) with minor modifications. Oligonucleotides were fluorescently labelled using DY-682. Labelling was performed by PCR using vector-specific DY-682-labelled primers followed by agarose gel extraction. Gel shifts were visualized using a LiCor Odyssey imaging system at 700 nm.

**Generation of transgenic lines**

The Gateway technology (Invitrogen) was used for generation of the overexpression and reporter constructs, using the entry vector pDONR221 and the destination vectors pK2GW7 (35S:SAUR10 overexpression) and pBGWFS7 (pSAUR10:GUS and pFUL:GUS) (Karimi et al., 2002). See Supplementary Table S2 for all primers. The FUL-VP16 line was generated by cloning a genomic fragment of the FUL locus, including 3.9 kb upstream region, which was fused in frame with the coding sequence of the strong activation domain of VP16 and followed by the CaMV 35S terminator, into the pBIN19 vector. Constructs were checked by sequencing, transformed into Agrobacterium strains LBA4404 or EHA105 and transformed to Col-0 plants using floral dip (Clough and Bent, 1998).

**Expression analysis**

RNA was extracted using the Invitrap® Spin Plant RNA Mini kit (Stratec Molecular) or with a CTAB/LiCl protocol. The RNA concentrations were adjusted to 200 ng/µl and a DNase treatment was performed using Ambion Turbo DNase (AM1907). For qRT-PCR analysis, the RNA was reverse transcribed using the iScript cDNA synthesis kit (BioRad) and the qRT-PCR reaction was performed with iQ SybrGreen supermix from BioRad. The quantitative RT-PCR analyses were performed on the BioRad iCycler. The UBC21 and/or TIP41 genes were used as reference genes (Czechowski et al., 2005).

**Hormone treatments and shade experiments**

For the hormone treatments, 10-day-old seedlings were removed from plates, containing 2.2 g/l Murashige and Skoog medium (MS), 10% sucrose and 0.8% agar, and incubated in liquid 2.2 g/l MS medium with or without hormones on the shaker for 4 h. The seedlings were floating with their roots submerged in the medium and their leaves contacting the liquid medium. The following hormone concentrations were used: 5 µM IAA (according to Bargmann et al., 2013), 5 µM brassinolide, 5 µM IAA + 5 µM brassinolide. After incubation, seedlings were frozen in liquid nitrogen and stored at −80°C prior to RNA isolation. To investigate the response to simulated shade, seedlings were grown on plates, containing 2.2 g/l MS, 10% sucrose and 0.9% agar, under control conditions for 12 d and then transferred to reduced R:FR for 4 h (see above) or kept for another 4 h under control conditions. To determine the branching phenotypes under simulated shade conditions, plants were placed under reduced R:FR conditions upon bolting and grown for another 2–3 weeks.

**Results**

**FUL binds many genes involved in hormone pathways, including SAUR10 and SAUR16**

The MADS domain transcription factor FUL is well known for its role in pistil and silique patterning (Gu et al., 1998; Ferrándiz et al., 2000b) and is expressed in a broad range of tissues (Supplementary Fig. S1). To identify direct targets of FUL, we initially focused on pistil and silique tissues, hereafter called silique, and performed a ChIP-Seq experiment with siliques from floral stages 12–16 (Smyth et al., 1990). This resulted in a list of 616 significantly enriched binding sites with a false discovery rate<0.01 (Supplementary Table S1), corresponding to 939 putative target genes with a peak within 3 kb upstream of the ATG and 1 kb downstream of the stop codon. This list showed a 65% overlap with loci identified for the putative FUL interaction partner SEP3 (De Folter et al., 2005; Kaufmann et al., 2009). The FUL ChIP list contains SHATTERPROOF2 (SHP2), a previously identified target of FUL in the silique (Ferrándiz et al., 2000b), but no enrichment was detected for INDEHISCENT (IND) another well described target of FUL (Liljegren et al., 2004), suggesting that the regulation of IND by FUL may be indirect.

To reveal the processes in which FUL predominantly acts, a gene ontology enrichment analysis was performed (Fig. 1A). Interestingly, the gene category with the highest enrichment in the FUL target set was ‘response to hormones’, directly followed by ‘response to abiotic processes’, indicating that FUL may play an important role in the crosstalk between developmentally and environmentally regulated processes. A closer inspection of the target list revealed in particular many auxin response genes, in addition to gibberelic acid (GA), cytokinin, ABA and ethylene pathway genes (Supplementary Table S1). Two highly enriched, closely related Small Auxin Upregulated RNA (SAUR) genes, SAUR10 and SAUR16, were identified, which had FUL binding sites in their promoters, approximately
SAUR10 contains a canonical CArG box, reported to be canonical (Fig. 1B). At the FUL binding positions, the promoter of 1380 bp and 1970 bp upstream of the start codon, respectively indicated with an asterisk. 

P <0.05) are shaped cauline leaves. See Supplementary Fig. S4 for additional phenotypes. Significant differences from the wild-type (Student's t-test, promoter fragment. (D) The overexpression phenotypes of the lines include longer siliques (picture stage 17B siliques) and differently response to abiotic stimulus; iii) regulation of cellular biosynthetic process; iv) regulation of primary metabolic process; and v) regulation of transcription, term finder was used for the analysis (http://go.princeton.edu/cgi-bin/GOTermFinder). The bars represent from left to right: i) response to hormones; ii) response to abiotic stimulus; iii) regulation of cellular biosynthetic process; iv) regulation of primary metabolic process; and v) regulation of transcription.

1380 bp and 1970 bp upstream of the start codon, respectively (Fig. 1B). At the FUL binding positions, the promoter of SAUR10 contains a canonical CArG box, reported to be commonly bound by MADS domain proteins (Kaufmann et al., 2009), while no clear CArG box was identified in the SAUR16 promoter (Supplementary Fig. S2). A second, smaller peak was identified in the SAUR10 promoter about 1200 bp upstream of the large peak, suggesting that SAUR10 could be regulated by a tetrameric FUL-containing complex that binds to two CArG boxes, as has been shown for MADS complexes involved in floral organ formation (Smaczniak et al., 2012; Theissen and Saedler, 2001). SAUR10 and SAUR16 belong to a clade of eight highly homologous SAUR genes, comprising SAUR8, SAUR9, SAUR10, SAUR12, SAUR16, SAUR50, SAUR51, and SAUR54 (Kodaira et al., 2011). The genes in this clade have not been functionally characterized yet, but both SAUR9 and SAUR50 have been reported to strongly inhibit PP2C-D activity (Spartz et al., 2014; Atamian et al., 2016), suggesting that proteins of the SAUR10 clade inhibit PP2C-Ds to induce cell expansion via modification of H+-ATPases, as has been reported for SAUR19 (Spartz et al., 2014).

To confirm that FUL is able to bind to the upstream regions of SAUR10 and SAUR16, we performed EMSA using the sequence below the peaks as probes (Fig. 1C, see Supplementary Table S2 for the probe sequences). A shift was clearly detected for the SAUR10 fragment, confirming that FUL can physically bind to this fragment. However, only a faint band indicating a shift was observed for the SAUR16 fragment, suggesting that FUL is not able to efficiently bind this fragment as a homodimer, in line with the lack of a canonical CArG box in this fragment. Possibly, FUL needs to interact with other transcription factors to strongly bind the SAUR16 upstream region. To investigate whether the CArG box in the SAUR10 fragment is essential for FUL binding, we also generated a probe in which the CArG box was disturbed by the mutation of AT to CG in the mid region of the motif (mSAUR10). We did not observe a shift for this fragment (Fig. 1C), confirming the importance of the CArG box for the binding of FUL. As FUL is able to bind strongly to sequences in the SAUR10 promoter in vivo and in vitro, we further focused on SAUR10 to unravel its function as direct target of FUL.

SAUR10 induces growth

To get an indication of the function of SAUR10, we generated an overexpression construct under control of the 35S promoter (35S:SAUR10) and transformed it to Col-0 Arabidopsis. The transgenic lines showed pleiotropic growth-related phenotypes, comprising of longer organs and tissues, such as sepals, filaments, etiolated hypocotyls, cauline leaves, pistils and siliques, and a wavy stem (Fig. 1D and Supplementary Fig. S3). These data indicate that SAUR10 can promote cell elongation similar to other SAURs (Chae et al., 2012; Spartz et al., 2012; Stamm and Kumar, 2013; Ren and Gray, 2015). As reported previously for SAUR36 (Hou et al., 2013), the leaves of the overexpression lines also senesced earlier than the wild-type leaves (Supplementary Fig. S3). We also tested different T-DNA insertion lines for SAUR10. However, the insertion in FLAG_590D09 could not be confirmed, while the insertion in SM_3_1724 was found to be located at the 3' end of the coding sequence and didn't affect the expression of SAUR10. The phenotypes of these lines were similar to the wild-type.

FUL is a pleiotropic regulator of plant growth and architecture

To investigate the role of FUL in the regulation of SAUR10 in the silique, we performed a quantitative RT-PCR experiment
(qPCR) to compare the expression of SAUR10 in wild-type and ful-7 (SALK_033647) mutant siliques from flower stages 11–14 (Smyth et al., 1990). However, we didn’t find an expression difference, suggesting that SAUR10 is not regulated by FUL in the siliques (Fig. 2A).

We therefore hypothesized that there could be other tissues in which FUL regulates SAUR10, possibly explaining some of the growth-related phenotypes observed in ful mutants (Fig. 2B–F). In addition to the well described phenotypes in the siliques and inflorescence meristem (Gu et al., 1998; Ferrándiz et al., 2000a), ful mutants also exhibit an altered cauline leaf shape (Fig. 2B, Gu et al., 1998). We also noticed distinct differences in stem development and architecture, including enhanced branching, decreased inflorescence branch angles, and shorter stem and internode lengths in ful-7 mutants (Fig. 2C–F and Supplementary Fig. S4). Shortly after bolting, wild-type stems elongate considerably, reaching a length on average of 12.8 cm (+/-5.4 cm) at 5 d after bolting (DAB), while ful-7 stems hardly elongated at that stage and are only on average 5.3 cm (+/- 2.8 cm, \( P < 0.001 \)) long. The difference in stem length is still visible at 10 DAB but disappears when the plants grow older (Fig. 2F and Supplementary Fig. S4).

35S:FUL overexpression lines exhibit a phenotype opposite to ful-7, with enhanced stem elongation and increased internode size in combination with reduced branch numbers and increased branch angles (Fig. 2B–F).

We compared the phenotypes of ful-7 mutants and wild-type plants to a pFUL:FUL-VP16 line (FUL-VP16). This line consists of a translational fusion of FUL with the strong transcriptional activation domain of the herpes virus protein VP16, driven by the FUL promoter. Genes that in wild-type plants are repressed by FUL are expected to become activated in this line. Phenotypes of ful mutants that are caused by target gene de-repression should thus be similar in the FUL-VP16 line, albeit probably to a lesser extent because FUL-VP16 has been generated in the Col-0 background and still contains an endogenous FUL copy that can repress the targets. The FUL-VP16 plants showed aberrant phenotypes that were probably a mix of enhanced target gene activation, for those targets that in wild-type tissues are activated by FUL, and activation of targets that are normally repressed by FUL. For example, FUL-VP16 siliques exhibited ‘shoulders’ and a short style similar to 35S:FUL siliques, while their overall phenotype more closely resembled that of ful-7.

Fig. 2. FUL is a pleiotropic regulator of plant growth and architecture. (A) Expression of SAUR10 in wild-type and ful-7 siliques from stages 12–16. (B) Cauline leaf phenotypes of Col-0, ful-7, 35S:FUL, and FUL-VP16 leaves (from left to right). (C) The number of branches formed along the main inflorescence. (D) Average internode length between the branches in plants at 10 DAB. (E) Average branch angle of all side branches along the primary stem of plants around 12 DAB. (F) Architecture phenotypes of Col-0, ful-7, 35S:FUL, and FUL-VP16 plants at 5 DAB (upper panel) and 10 DAB (lower panel). In (A), the error bars represent the SE based on two biological replicas. In (C–E), the error bars represent the SE based on at least 20 measurements. Significant differences from the control (Student’s t-test, \( P < 0.05 \)) are indicated with an asterisk.
siliques (Supplementary Fig. S5A). To verify that FUL-VP16 is not co-suppressing endogenous FUL, we tested FUL transcript abundance in stem and branches and found a 1.5–2-fold higher expression of FUL (Supplementary Fig. S5B), indicative of the presence of an additional FUL copy (FUL-VP16) and not of co-suppression. Several FUL-VP16 phenotypes, including stem and cauline leaf growth, were more similar to 35S:FUL than to ful-7, suggesting that these traits are largely regulated by activation of target genes in the wild-type (Fig. 2B, F).

**FUL represses SAUR10 in the stem**

To investigate whether FUL can regulate SAUR10 in tissues other than the silique, we generated reporter lines for SAUR10 (pSAUR10:GUS) and crossed these into the ful-7, 35S:FUL and FUL-VP16 backgrounds. The GUS expression patterns of the pSAUR10:GUS lines was rather specific, with staining predominantly in the veins and petioles of rosette leaves and cauline leaves. In the context of the flower, expression appeared only in stage 12 flowers in the vasculature of the style, and in stage 13 flowers in the apical parts of the stamen filaments and petals. Apart from the expression in the style, no expression was observed in the pistil or silique (Fig. 3A).

SAUR10 expression in ful-7 and FUL-VP16 stems was clearly different from expression in wild-type plants. In wild-type inflorescences, pSAUR10:GUS expression was not, or only very weakly, observed in the stem. However, in both ful-7 and FUL-VP16 inflorescences, GUS expression was present in the upper stem region, indicating that SAUR10 is de-repressed.

![Fig. 3. SAUR10 is regulated by FUL in stems. (A) GUS staining pattern in different organs and tissues of a pSAUR10:GUS line. JRL, juvenile rosette leaf; ARL, adult rosette leaf; CL, fully expanded cauline leaf; Fl, flower stage 13; Si, silique stage 17. (B) GUS staining pattern of pSAUR10:GUS inflorescence stems from different FUL backgrounds. (C) Relative expression of SAUR10 in the upper stem segment of Col-0, ful-7, 35S:FUL, and FUL-VP16 plants 8–10 DAB. (D) Graph from a ChIP-qPCR experiment showing the enrichment of the SAUR10 fragment relative to two reference sequences in a ChIP sample from stem tissue. The enrichment of the fragments was calculated as a percentage of the input sample. Error bars represent the SE of three biological replicas in the case of expression analyses, and two replicas for the ChIP-PCR. Significant differences from the control (Student’s t-test, P<0.05) are indicated with an asterisk.](image-url)
in ful-7 and activated by FUL-VP16 in inflorescence stems (Fig. 3B). To investigate whether SAUR10 transcript levels were in line with these results, RNA was extracted from a 1 cm stem segment directly below the inflorescence meristem, and qPCR was performed. SAUR10 expression was found to be approximately 10-fold higher in ful-7 and 6-fold higher in FUL-VP16, while the levels in 35S:FUL were not significantly decreased, probably because SAUR10 expression is already very low in wild-type stems (Fig. 3C). These data are consistent with direct repression of SAUR10 by FUL, and are in line with our observation that pFUL:GUS is active in the inflorescence stem (Supplementary Fig. S1).

Since our ChIP-Seq experiment was performed with silique tissue, we were not certain if FUL could directly bind to SAUR10 in the stem. To test this, we performed ChIP-qPCR experiments using stem tissue of primary and secondary inflorescences. We found a distinct enrichment for SAUR10 in both replicates (Fig. 3D), showing that FUL can directly bind to the SAUR10 locus in stems as well. In conclusion, we show here that FUL can directly repress SAUR10 expression in the stem.

Auxin and BR induce SAUR10 expression synergistically

Experiments with auxin have identified SAUR10 as one of the Arabidopsis SAUR genes clearly upregulated in response to auxin treatment in seedlings (Goda et al., 2004; Paponov et al., 2008; Chapman et al., 2012; Bargmann et al., 2013). SAUR10 has also been reported to be responsive to brassinosteroids (BRs) (Yu et al., 2011). To investigate the interaction between FUL-controlled repression and hormone-induced upregulation, we treated wild-type, ful-7, 35S:FUL, and FUL-VP16 seedlings with auxin, namely 5 µM indole-3-acetic acid (IAA), and/or BRs, namely 5 µM brassinolide (BL), for 4 h. Wild-type seedlings treated with BL showed a 4-fold induction of SAUR10 compared with mock-treated seedlings, while the seedlings treated with auxin showed a 10-fold increase, confirming the previously published hormone responses (Fig. 4A). Interestingly, a combination of IAA and BL resulted in a synergistic effect on the induction of transcription and led to an impressive 65-fold higher expression of SAUR10 in seedlings. In all treatments, the expression of FUL did not change, while the GUS transcript levels in treated pSAUR10:GUS plants showed a response similar to SAUR10, indicating that hormone induction is regulated by the promoter rather than through post-transcriptional mechanisms. To test whether FUL could influence the hormone-induced increase in SAUR10 expression, we performed the IAA-BL treatments in ful-7, 35S:FUL, and FUL-VP16 seedlings. This revealed no significant differences (Fig. 4B), suggesting that FUL does not influence the hormonal upregulation of SAUR10 in seedlings.

The response of SAUR10 to shade is influenced by FUL

SAUR10 can be highly induced by a combination of auxin and BRs, two hormones that have together been associated with shade responses (Pierik et al., 2009; Keuskamp et al., 2011). When exposed to shade, plants sense a decreased R:FR ratio, as well as depletion of blue light, and respond...
by phenotypic changes such as stem, internode and petiole elongation, and hyponastic leaf movement, together referred to as the shade-avoidance syndrome (SAS). To investigate whether SAUR10 is responsive to simulated shade, we transferred 12 day-old seedlings to low R:FR light conditions and compared the expression of the genes with seedlings from the control condition. SAUR10 showed a marked increase of expression after 4 h of low R:FR, indicating that it can indeed positively respond to shade. This response was not different in ful or FUL-VP16 seedlings, but was significantly reduced in 35S:FUL seedlings, suggesting that the ectopic overexpression of FUL represses the shade-induced upregulation of SAUR10 (Fig. 4C).

To investigate whether the effect of FUL was more distinct in the tissue where it actually represses SAUR10, we transferred wild-type, ful, and 35S:FUL plants to low R:FR conditions and harvested stem segments after 4 h. The expression in wild-type stems was upregulated four times compared with control stems, while the upregulation in ful mutant stems increased to 14 times (Fig. 4D). The upregulation in ful stems is higher than can be explained by an additive effect of de-repression and shade-induced upregulation, suggesting that loss-of-FUL allows a greater response to the light conditions. No significant upregulation compared with control conditions could be detected at all in 35S:FUL stems (Fig. 4D), pointing to a much stronger effect of FUL repression in the regulation of SAUR10 expression in the stem than in seedlings. In conclusion, these experiments show that SAUR10 is a distinct responder to both hormone and light stimuli, and that the light response can be attenuated by FUL in the stem.

De-repressed SAUR10 expression correlates with longer cells in the stem

We inspected the stem and architecture phenotype of the ful mutants further to identify phenotypic features that could be attributed to SAUR10 de-repression. Given the longer organ phenotype of the SAUR10 overexpression lines, we expected the inflorescence stem of ful-7 plants, in which SAUR10 is more highly expressed, to be longer than the wild-type, and that of 35S:FUL to be shorter. However, we found the opposite effect in young inflorescences, which were shorter in ful-7 plants, with a significantly smaller distance between side branches (Fig. 2D) and silique internodes (Supplementary Fig. S4). To determine whether the cell sizes in the stem were in line with the internode sizes, we measured cell length in wild-type, ful-7, and 35S:SAUR10 stems between internodes one and two. Interestingly, this revealed longer cells in the ful-7 and 35S:SAUR10 stems compared with wild-type (Fig. 5A, B), showing that ful-7 stems have longer cells despite having shorter internodes. Thus, the upregulation of SAUR10 in the ful-7 stem probably does result in longer cells, but the shorter stem phenotype is caused by reduced cell division as a result of de-regulation of other target genes.

The ful mutant stem phenotype is caused by a combination of de-regulated genes

FUL represses SAUR10 in the stem, but the stem phenotype of the ful-7 line, which shows retarded cell division, indicates that FUL additionally activates other targets to regulate stem growth and architecture. We therefore examined the FUL ChIP target list in more detail for genes that have been associated with growth responses. In addition to genes involved in cytokinin and auxin signalling, such as the cytokinin degradases CKX5, CKX6, and CKX7, and genes encoding the AUX/IAA proteins IAA8 and IAA16, we also found a remarkable number of genes that are implicated in the light-sensitive growth of hypocotyls, encoding transcription factors involved in the BZR-PIF-ARF-DELLA pathway (Bai et al., 2012; Oh et al., 2014). These include the PIF genes PIL1, PIL2, and PIL4, the DELLA genes RGL1, RGL2, and GAI, the BR pathway genes BZR1 (only one replica), BZR2, and BRASSINOSTEROID INSSENSITIVE 1 (BIN1), and also the photoreceptor phytochrome A (PHY) and its targets PHOTOCROME RAPIDLY REGULATED 1 (PAR1) and PAR2, which are negative regulators of the shade response and reduce the expression of several SAURs (Roig-Villanova et al., 2007).

To determine if any of these genes were regulated by FUL in the stem, we performed qPCR analysis to compare the transcription levels of ful-7 mutant stems with wild-type. We detected significantly lower transcript levels for BIN1, PIL1, RGL2, GAI, and PAR2, indicating that FUL activates these genes in wild-type stems (Fig. 5C). We selected these targets to test whether they were also bound by FUL in the stem, and found enrichment for all five tested genes in two independent ChIP experiments (Fig. 5D). However, the decreased expression of these genes will have an effect on cell elongation rather than on cell division (Nam and Li, 2002; Salter et al., 2003; Roig-Villanova et al., 2007; Li et al., 2012) and can thus not entirely explain the ful stem phenotype. CKX5, CKX6, and CKX7, on the other hand, are cytokinin oxidase/dehydrogenase (CKX) genes, which can catalyze the degradation of cytokinins and have been reported to determine the activity of the shoot meristem. We found that the transcript levels of CKX5 and CKX6 are significantly decreased in ful-7 stems, while upregulated in 35S:FUL stems (Fig. 5E). This would lead to reduced cytokinin breakdown in ful mutants and a higher meristem activity (Werner et al., 2003; Bartrina et al., 2011), which could explain the increased branching and shorter internodes as observed. We also tested whether CKX5 and CKX6 were bound by FUL in the stem and detected a clear enrichment for the CKX5 and CKX6 loci compared with two reference loci (Fig. 6D). FUL thus appears to regulate a complex network of genes that are likely to have opposite functions in stem growth. The outcome of this regulation probably depends on other factors that interfere with this network, such as hormone concentration and light quality.

De-repressed SAUR10 expression correlates with decreased branch angles

We also noticed that the branch angles in ful-7 and FUL-VP16 plants are significantly smaller than in the wild-type, while being larger in 35S:FUL (Figs 2E and 6A). Branch angle is highly dynamic and depends on gravitropic and phototropic signals, both of which depend on auxin gradients (Roychoudhry et al., 2013; Liscum et al., 2014). In addition,
BRs have also been found to influence auxin-mediated phototropic responses (Liscum et al., 2014). We therefore investigated whether the altered branch angles in ful mutants could be correlated to SAUR10 expression. pSAUR10:GUS activity was specifically observed at the abaxial side of young Col-0 branches and sectioning revealed that expression was located in the abaxial epidermal layer (Fig. 6B, C), pointing to a role for SAUR10 in branch bending. Since directed auxin-induced hypocotyl/stem elongation occurs in accordance with the SAUR-mediated acid growth theory and growth is predominantly regulated by the epidermis (Fendrych et al., 2016; Procko et al., 2016), we reasoned that SAUR expression in the abaxial epidermal cell layer is expected to enhance cell elongation on this side, resulting in more vertical growth of the branch (Fig. 6D). To determine if auxin concentrations are higher at the abaxial side of the branch, we examined the DR5:GUS auxin reporter line (Ulmasov et al., 1997). This revealed a weak GUS signal in DR5:GUS branches, specifically at the abaxial side after prolonged staining (Fig. 6E), indicating that auxin levels are indeed higher at the abaxial side of the branch, probably inducing SAUR10 expression.

Similar to the de-repression of SAUR10 in the primary inflorescences of ful-7 and FUL-VP16 plants, SAUR10 was also clearly upregulated in the stem of the branch inflorescences. We examined the pSAUR10:GUS pattern in detail in the ful-7 samples compared to the wild-type and found that in young, emerging branches, the GUS signal was higher than in the wild-type and could also be observed at the adaxial side (Fig. 6F). In contrast, pSAUR10:GUS was rarely observed in wild-type branches at this stage (Fig. 6F). In accordance with FUL repressing SAUR10 in branches, the pSAUR10:GUS signal was mostly absent in 35S:FUL branches (Supplementary Fig. S6A). These pSAUR10:GUS data were
confirmed by qPCR analysis of *SAUR10* transcript levels in branches 4–6 DAB, which revealed a 3–4-fold higher expression in *ful-7* and a 5-fold reduction in *35S:FUL* (Fig. 6G). The higher abaxial expression in the region below the *ful-7* (Fig. 6F) and FUL-VP16 (Supplementary Fig. S6A) inflorescences can well explain the more vertical branching in these lines. The expression of *FUL* itself also corresponds with the observed *SAUR10* expression, as *FUL* is highly expressed throughout branches and exhibits the highest levels just below the inflorescences (distal part, Fig. 6H). This is in line with the observation that *SAUR10* de-repression is most prominent in this region.

Abaxial *SAUR10* expression is affected by light conditions

To investigate whether the expression of *pSAUR10:GUS* in branches was sensitive to light conditions, we reduced the R:FR ratio and determined the GUS pattern in the inflorescences after 24 h. We observed a relocation of the GUS signal to both sides of the branch (Fig. 6I), consistent with a positive response of *SAUR10* to the shaded, low R:FR conditions at both the adaxial (Ad) and abaxial (Ab) sides and thus an increase of the Ad/Ab ratio. In line with this result, plants that were exposed for a longer period to reduced R:FR conditions exhibited substantially increased branch angles (Supplementary Fig. S7A, B), suggesting a more homogeneous growth factor distribution. If *SAUR10* is indeed regulating branch bending, branch angle should also be disturbed in *35S:SAUR10* plants, where *SAUR10* is ectopically expressed at the adaxial side. The expected increased Ad/Ab ratio would then result in more horizontal branch growth. Indeed, we observed larger branch angles in the *35S:SAUR10* line, although branch growth was highly variable and irregular (Supplementary Fig. S7A, B). This irregular growth was even
stronger under reduced R:FR conditions (Supplementary Fig. S7B), suggesting that the SAUR10 overexpression phenotype is enhanced by the SAS response.

We also tested to what extent FUL could influence the effect of simulated shade on SAUR10 expression levels and branch angle phenotype. In branches of the ful mutant, de-repression of SAUR10 combined with shade-induced expression resulted in an almost 30-fold upregulation of SAUR10 in young branches (Fig. 6J). This is again more than can be explained by an additive effect alone, suggesting that the absence of FUL allows an enhanced response to the light conditions, causing an increase in expression on both the abaxial and adaxial sides. In line with this, simulated shade resulted in more horizontal branch growth in all backgrounds (Supplementary Fig. S6B).

In conclusion, we found SAUR10 to be abaxially expressed in branches, presumably as a result of auxin accumulation at the shaded side of the branch. The de-repression of SAUR10 in the ful mutant results in increased abaxial expression in the distal part of the branch, which can cause the increased bending of the branches observed in ful mutants. In wild-type branches, FUL represses SAUR10, thereby possibly preventing over-bending of the branch and attenuating responses to light conditions.

Discussion

We show here that the MADS domain transcription factor FUL is a pleiotropic regulator of plant development, which plays important roles in plant growth and architecture in addition to its well known functions in fruit development and flowering time. In particular the deviating branch angles in the ful mutants are very interesting, since little is known about this trait, which is particularly important for crop yield. Loci in other species that could be linked to branch angle have been associated with the auxin pathway, such as the LA1 locus in rice (Li et al., 2007) and the GRETCHEN HAGEN 3 (GH3) gene in Brassica napus (Liu et al., 2016). Our analysis also indicates that members of the SAUR family can play an important role in branch bending, especially in response to environmental conditions like high plant density. We demonstrate that SAUR10 is specifically expressed at the abaxial side of the branch, thereby affecting branch angle. Enhanced and prolonged abaxial expression of SAUR10 in the ful mutant can explain its more vertical branching phenotype. The activity of SAUR10 in stems and branches appears to be regulated by interplay between hormone-induced upregulation and FUL-controlled repression. In addition, our data reveal that FUL directly regulates a number of other genes involved in hormone and light signalling, of which the de-regulation contributes to the ful mutant phenotype. To what extent these genes contribute to the ful mutant phenotype needs to be further investigated. However, a picture is emerging that FUL can regulate plant growth and architecture in concert with the environment by balancing the expression of hormone and light responsive factors. It will be interesting to study how the expression of FUL changes during plant development, and if for example, older plants are less responsive to environmental signals through increased FUL expression.

FUL interacts with the IAA/BR pathway to repress SAUR10

Despite the broad expression pattern of FUL, de-repression of SAUR10 in the ful mutant only occurs in a limited number of tissues, indicating that SAUR10 activation requires additional tissue-specific factors, such as high auxin and BR levels. In addition to repressing SAUR10 under control conditions, we also found that FUL can buffer the hormone- or shade-induced expression of SAUR10 in stems and branches, suggesting that FUL can attenuate the activity of the auxin and/or BR response transcription factors. Since SAUR10 has been identified as a direct target of both ARF6 and BZR1 by ChIP-Seq analyses (Oh et al., 2014), it is possible that FUL can interact with either or both of these factors, thereby repressing transcription. FUL binds to a CArG box in the SAUR10 promoter, which is located 260 bp upstream of a canonical ARF binding motif and only 100 bp downstream of an AuxRE-related element identified by Walcher and Nemhauser (2012). Binding of FUL to the CArG box may disturb the interaction between ARF6 and BZR1 (previously reported by Oh et al., 2014). Our results indicate that FUL is not required to determine the SAUR10 expression domain, but rather to fine-tune or buffer the response to hormonal stimuli.

SAURs integrate environmental, hormonal, and developmental signals in the growth response

Different studies in Arabidopsis, soybean and maize have identified SAUR genes as hormone-responsive growth regulators. However, SAURs have also been found as direct targets of several transcription factors functioning in plant development, suggesting that they function downstream of both developmental and hormonal regulators to direct plant growth. We demonstrate here that SAUR10 is regulated by hormonal stimuli, light signals, as well as by the developmental regulator FUL, and can thereby integrate a plethora of signals in the growth response. Several recent reports have strengthened the idea that SAURs can in general respond to a variety of upstream factors, integrating these in the regulation of cell elongation through interaction with PP2C-Ds (Spartz et al., 2014; Challa et al., 2016; Procko et al., 2016; Sun et al., 2016). This has been most thoroughly investigated in seedlings, where SAUR genes have been grouped according to their response to light conditions in hypocotyls and cotyledons (Sun et al., 2016), indicating that the growth response is controlled by a cluster of similarly regulated SAURs, rather than by single genes. In that respect, SAUR10 may not be the only SAUR with differential expression between the abaxial and adaxial side of the Arabidopsis branches. Interestingly, a SAUR50-like gene - SAUR50 belongs to the SAUR10-clade - has recently been identified to be responsible for heliotropism in sunflower (Atamian et al., 2016). The SAUR50-like gene is expressed more highly on the east side of the stem during
the day, enabling the shoot apex to move gradually from east to west along with the sun. In addition, several other SAURs have been reported to be responsive to shade (Roig-Villanova et al., 2007; Spartz et al., 2012; Procko et al., 2016), indicating that the dynamic response to shade may to a large extent be executed by SAUR proteins. Differential expression of SAUR genes may thus in general allow directional growth in a variety of species.

**Supplementary data**

Supplementary data are available at JXB online.

Table S1. Identified loci with significant enrichment in the FUL ChIP-Seq

Table S2. Primer list.

Fig. S1. FUL is widely expressed in Arabidopsis.

Fig. S2. Binding sites of FUL in the SAUR10 and SAUR16 upstream regions.

Fig. S3. Phenotypes of the SAUR10 overexpression lines. Fig. S4. Distance between the silique internodes.

Fig. S5. Characterization of the FUL-VP16 plants.

Fig. S6. FUL represses SAUR10 in branches, which can be correlated to branch angle.

Fig. S7. The architecture of Col-0 and 35S:SAUR10 plants changes under reduced R:FR conditions.

**Data deposition**

ChIP-Seq data. Gene Expression Omnibus (GEO). Accession number GSE79554.

https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE79554

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